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The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.

Tamoxifen Versus Ethinyl Estradiol in the Treatment of Postmenopausal Women With Advanced Breast Cancer[1]

L. Beex,* G. Pieters, A. Smals, A. Koenders, T. Benraad, and P. Kloppenborg[2]

SUMMARY

In a randomized study, 63 postmenopausal patients with advanced breast cancer were treated with ethinyl estradiol (EE_2) or the antiestrogen tamoxifen to compare the efficacy and side effects of both drugs. EE_2 was always given in combination with chlorothiazide to prevent fluid retention. Pretreatment characteristics of the patients of both groups did not differ significantly. Objective remissions were achieved in 31% of the EE_2-treated patients and in 33% of the tamoxifen-treated patients. The median duration of remission was 12 months (range, 5–32) for the EE_2 group and 11 months (range, 5–26) for the tamoxifen group ($P > 0.1$), and the estimated median survival times from the start of treatment were 31 and 25 months, respectively ($P > 0.1$). The best treatment results in both groups were obtained in patients with estradiol receptor-positive tumors and less advanced disease. After therapy was stopped, objective withdrawal responses were observed in EE_2- but not in tamoxifen-treated patients. Two patients receiving EE_2 had to discontinue treatment because of drug-related liver function impairment. Both patients had cholelithiasis. Two patients in the tamoxifen-treated group discontinued therapy because of nausea. Deep venous thrombosis occurred in one patient receiving EE_2, whereas two patients receiving tamoxifen developed superficial thrombophlebitis. Other side effects in both groups of patients, including initial hypercalcemia, were mild. It is concluded that both treatment regimens, EE_2 or tamoxifen, are equally effective with respect to induction and duration of remission in postmenopausal patients with advanced breast cancer. Side effects of EE_2 therapy appeared to be more serious than those of tamoxifen treatment.

[Cancer Treat Rep 65:179–185, 1981]

High doses of conjugated estrogens have proven to be effective in about one third of postmenopausal patients with advanced breast cancer (1,2). However, the therapeutic use of these hormones is limited by a considerable number of side effects (3).

Antiestrogens seem to be at least as effective as high doses of estrogens in the treatment of such patients (4–6), and among these drugs, tamoxifen (Nolvadex) has been the most extensively investigated. Side effects were infrequent and usually mild (7).

In this paper, the results of treatment with ethinyl estradiol (EE_2) (Lynoral) and tamoxifen are compared with regard to induction and duration of remission, withdrawal reactions, survival times, and side effects.

PATIENTS AND METHODS

Postmenopausal patients with histologically proven metastasized breast cancer and measurable disease

[1]Received Mar 14, 1980; revised June 10, 1980; accepted June 24, 1980.

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were eligible for this study. Menstrual cycles had to have ceased at least 2 years previously, either spontaneously or after ovariectomy, and previous treatment could not include the drugs under investigation. Patients with rapidly progressive disease or with central nervous system or massive liver involvement were excluded from this study.

Patients were randomized to receive EE_2 or tamoxifen. EE_2 was given in an initial dose of 0.5 mg daily, increasing within 5 days to 3 mg daily. This treatment was always combined with chlorothiazide (500 mg daily) to prevent fluid retention. Patients with congestive cardiac disease were excluded from EE_2 treatment and received tamoxifen (three). Tamoxifen was prescribed in a dose of 20 mg twice daily. The drugs were given for at least 2 months before they were considered to have failed. In case of remission, treatment was continued until relapse. After discontinuation of EE_2 or tamoxifen, patients were observed for at least 2 months to assess a potential withdrawal response. The duration of this observation period was limited by the progression rate of the disease. Subsequent therapy of patients with slowly progressing disease consisted of crossing over from tamoxifen to EE_2 or vice versa. Otherwise, or if there was progression during crossover treatment, patients received 5-FU combined with tamoxifen or cyclophosphamide, methotrexate, and 5-FU combined with tamoxifen (CMFT); treatment with 5-FU and tamoxifen sometimes was followed by CMFT therapy. Further palliative treatment consisted of combination chemotherapy with doxorubicin and vincristine or cyclophosphamide and of aminoglutethimide, androgens, corticosteroids, and/or local radiotherapy. Results of therapy were assessed according to the criteria of the International Union Against Cancer (8), by at least two physicians and one radiologist (Department of Radiology; Prof. Dr. W. Penn, head). The extensiveness of metastatic disease has been recorded arbitrarily as "one localization" (ie, one single localization of metastasis, or in the case of skin lesions, one or more localizations close together), "multiple sites" (ie, two to five metastatic lesions or skin metastases in different parts of the body), and "very advanced disease" (ie, more localizations than in the categories mentioned above).

The duration of remissions and the survival time functions were calculated by the method of Kaplan and Meier (9). Differences between such functions have been tested by the nonparametric test developed by Gehan and Mantel (9). Further statistical analysis was performed by Wilcoxon's two-sample test and Fisher's chi-square test. Estradiol receptor (ER) activity determinations were carried out by incubating cytosol fractions of tumor tissue with different concentrations of tritiated steroid Nonspecific binding was determined in the presence of excess diethylstilbestrol. Bound and free steroids were separated by dextran-coated charcoal, and results were calculated according to Scatchard. Cytosols showing no saturable high-affinity binding were considered as ER-negative (10). The lower limit of sensitivity of this assay appeared to be 48 fmols/ml of cytosol. In our laboratory, the lowest ER value measured in human breast tumor cytosol was 5 fmols/mg of protein. Tissue for ER analysis was obtained from skin or lymph node metastases (28 patients) and occasionally from the primary tumor (six), pelvic lesions (two), or bone metastases

TABLE 1.—Pretreatment characteristics

Characteristics*	EE_2 group (29 patients)	Tamoxifen group (30 patients)
Disease-free interval (DFI)		
Patients without DFI	5	3
Patients with DFI	24	27
Median duration in mos	24	36
Range in mos	5–173	8–360
Age in yrs		
Median	63.0	63.5
Range	54–77	44–82
Menopausal period† in yrs		
Median	11.0	15.0
Range	4–31	2–32
Dominant localization of metastasis		
Soft tissue	4	5
Bone	13	10
Viscera	12	15
Extensiveness of disease		
One localization	6	8
Multiple sites	20	19
Very advanced	3	3
ER activity		
Positive	11	13
Negative	7	7
Unknown	11	10
Previous treatment		
None	22	21
Cytostatic drugs	3	6
Therapeutic ovariectomy	0	1
Androgens	4	2
Interval between diagnosis of advanced disease and start of treatment in mos		
Median	2	3
Range	0–99	0–70

*Unless otherwise indicated, values = No. of patients.
†Postovariectomy in 3 patients of each treatment group.

(two). In most patients, ER activity determinations were done within 6 months prior to the start of hormonal treatment.

RESULTS

Response

Thirty-one patients received EE_2 and 32 received tamoxifen. In two patients of each group, treatment was stopped within 8 weeks from the start of therapy because of nausea (tamoxifen) or liver function impairment (EE_2). Pretreatment characteristics of the remaining patients are summarized in table 1. The groups did not differ significantly in age, duration of the disease-free interval or the menopausal period, localization of the dominant metastasis, extensiveness of advanced disease, ER status, previous therapeutic regimens, or time interval between the diagnosis of advanced disease and the start of treatment with EE_2 or tamoxifen.

Thirty-one percent (nine of 29) of the patients treated with EE_2 and 33% (ten of 30) of the patients treated with tamoxifen achieved an objective remission. Furthermore, in two patients of each group, progressive disease before treatment remained stationary for 6–36 months during therapy. Objective responses in both groups occurred only in ER-positive or ER-unknown patients but not in ER-negative patients (table 2). No objective remissions were achieved in patients with very advanced disease (table 2).

When patients were divided into subgroups according to age, duration of menopausal period, duration of disease-free interval, dominant localization of metastasis, progression of disease, ER activity in tumor tissue, and previous treatment, no significant differences in rates of response to treatment with EE_2 or tamoxifen were found (table 2).

TABLE 2.—Prognostic factors and response to treatment*

Factor	EE_2 group	Tamoxifen group	Total
ER activity			
Positive	5/11	7/13	12/24†
Negative	0/7	0/7	0/14†
Unknown	4/11	3/10	7/21
Extensiveness of disease			
One localization	4/6	4/8	8/14‡
Multiple sites	5/20	6/19	11/39‡
Very advanced	0/3	0/3	0/6‡
Age			
≤ 60 yrs	4/11	2/12	6/23
> 60 yrs	5/18	8/18	13/36
Duration of menopausal period			
2–10 yrs	3/11	2/12	5/23
> 10 yrs	6/18	8/18	14/36
Disease-free interval			
No interval	0/5	2/3	2/8
≤ 1 yr	0/5	1/3	1/8
> 1 yr	9/19	7/24	16/43
Dominant localization of metastasis			
Soft tissue	1/4	2/5	3/9
Bone	4/13	2/10	6/23
Viscera	4/12	6/15	10/27
Previous treatment			
None	7/22	7/21	14/43
Chemotherapy	1/3	2/6	3/9
Ovariectomy	0/0	0/1	0/1
Androgens	1/4	1/2	2/6

*Values = No. of objective remissions/No. of patients.
†ER-positive vs ER-negative: $\chi^2 = 8.047$, DF = 1, $P < 0.01$.
‡One localization vs multiple sites and very advanced disease combined: $\chi^2 = 3.840$, DF = 1, $P = 0.05$.

Duration of Remission, Withdrawal, Responses, and Subsequent Therapy

Figure 1 shows the estimated duration of remission for both groups of patients. The median duration of remission for the EE_2-treated patients was 12 months (range, 5–32), with one patient still in remission after 23 months. The median duration of remission for the tamoxifen-treated patients was 11 months (range, 5–26), with one patient still in remission after 11 months ($P > 0.1$). Objective withdrawal responses, although not fulfilling criteria of objective remision, were found in four of 18 EE_2-treated patients but in none of 13 tamoxifen-treated patients. Patients with objective withdrawal responses were not necessarily responders to the first therapy. Crossing over from EE_2 or tamoxifen resulted in objective remissions in one of three patients previously responding to EE_2, in two of four with no prior response to EE_2, in one of three previously responding to tamoxifen, and in none of two previously not responding to tamoxifen. Types and number of subsequent therapies did not differ significantly between groups (table 3).

Survival

As can be expected, survival functions of patients responding to therapy were significantly better than those of patients who failed therapy (fig 2). Figure 2 also shows that there were no significant differences between survival functions of patients treated with either EE_2 or tamoxifen. The estimated median survival times from start of treatment for all patients receiving EE_2 or tamoxifen were 31 months (range, 3 to > 60) and 25 months (range, 4 to > 42, respectively) ($P > 0.1$).

Side Effects and Toxicity

Table 4 summarizes the side effects and toxicity of both drugs. Of 31 patients treated with EE_2, three showed apparently drug-related liver function impairment. Those patients proved to have cholelithiasis. Liver functions returned to normal in one patient without withdrawal of EE_2 and in two patients after (or because of) discontinuation of this drug. Both patients subsequently received tamoxifen without serious side effects. Of 32 patients initially receiving tamoxifen, two discontinued treatment within 4 weeks from start of treatment because of persisting nausea. In all other patients nausea or vomiting was transient and more pronounced in the EE_2 group.

Aggravation of hot flushes was noted only by patients on tamoxifen treatment.

Transient drug-related hypercalcemia occurred in three patients and was successfully treated with hydration and furosemide. It was not necessary to

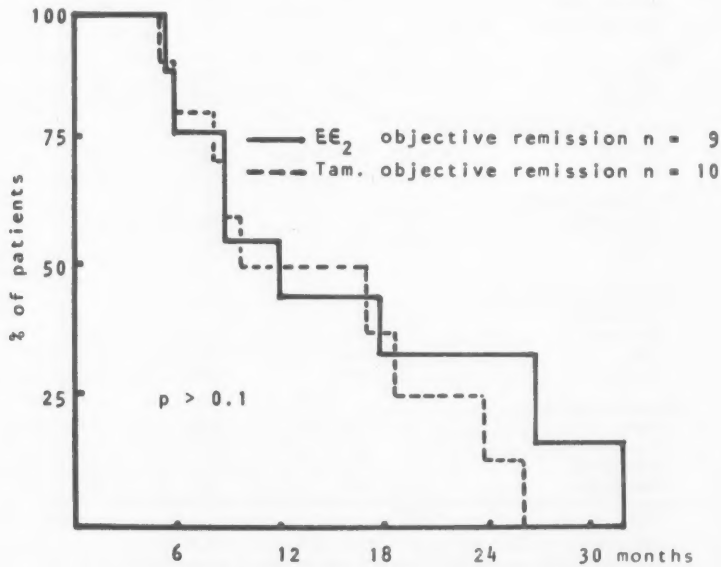

FIGURE 1.—Estimated duration of remission. Tam. = tamoxifen.

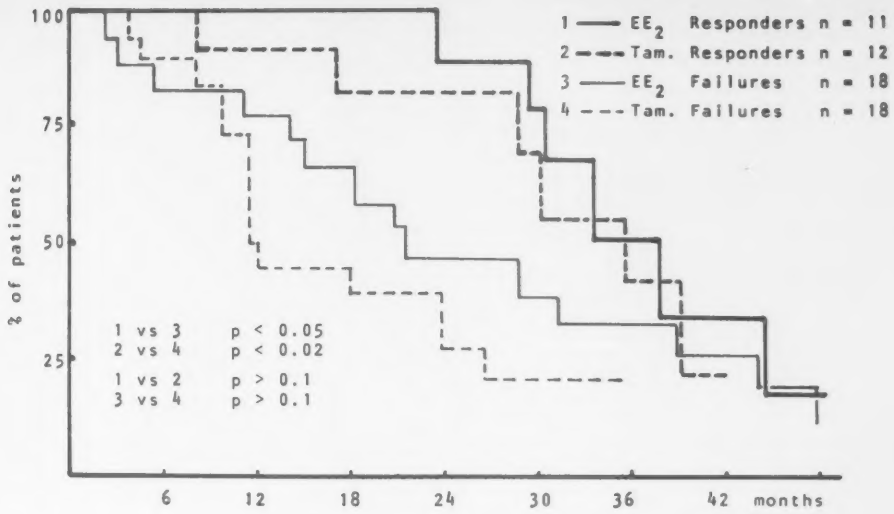

FIGURE 2.—Estimated survival time from start of treatment. Tam. = tamoxifen.

TABLE 3.—Subsequent therapy*

Therapy	EE_2 group	Tamoxifen group
5-FU + tamoxifen	12	5
CMFT	14	13
Doxorubicin in combination	7	5
Aminoglutethimide	1	4
Other	3	2

*Values = No. of patients treated. $\chi^2 = 4.347$, DF = 4, $P > 0.1$.

TABLE 4.—Side effects and toxicity*

Side effect	EE_2 group	Tamoxifen group
Nausea or vomiting	14	8†
Liver function impairment	3†	0
Hot flushes	0	4
Initial increase of pain	2	4
Initial hypercalcemia	1	2
Vaginal spotting	7	0
Vulvovaginitis	0	3
Withdrawal bleeding	All	None
Spontaneous galactorrhea	1	0
Edema	4‡	1

*Values = No. of patients.
†Requiring discontinuation of treatment in 2 patients.
‡Despite treatment with chlorothiazide.

stop EE_2 or tamoxifen therapy in any of these patients. Initial increase of tumor pain was observed in both groups of patients. Initial hypercalcemia and/or initial increase of tumor pain were not seen in the patients with ER-negative tumors.

Vaginal spotting and withdrawal bleeding were observed only in patients receiving EE_2 treatment, whereas vulvovaginitis due to an atrophic mucosa occurred only in patients receiving tamoxifen therapy. Almost all patients receiving EE_2 noted some increase in the volume of the healthy breast, as could be verified by repeated mammography. This was not observed in the group receiving tamoxifen. One patient receiving EE_2 showed spontaneous galactorrhea.

Fluid retention was observed in four patients receiving EE_2 and in one patient receiving tamoxifen. No significant alterations of body weight were observed in the remaining patients, but it has to be stressed that all patients receiving EE_2 treatment simultaneously received chlorothiazide. No significant alterations in mean arterial blood pressure were found in patients of either group.

One patient receiving EE_2 therapy developed calf thrombosis and nonfatal lung embolism. This complication was observed during the period she was in remission from lung metastasis. Two patients of the tamoxifen group developed a possibly drug-related superficial phlebitis of the leg.

No systematic alterations in kidney or bone marrow functions were observed in the patients during treatment with EE_2 or tamoxifen.

DISCUSSION

In the present study, no difference could be demonstrated in the antitumor effects of EE_2 or tamoxifen in postmenopausal patients with advanced breast cancer. These results agree with preliminary data of Stewart (11) comparing the efficacy of diethylstilbestrol and tamoxifen in similar groups of patients. Remission rates and duration in the present study correspond well to those in the literature (1,2,6,7).

Both EE_2 and tamoxifen failed to induce objective tumor regressions in patients with ER-negative tumors, whereas about 50% of patients with ER-positive tumors showed a favorable response. These figures agree with data in the literature (7,12).

Another important discriminating factor for predicting the results of both therapies was the extensiveness of metastatic disease: patients with very advanced disease did not respond to EE_2 or tamoxifen. It has to be stressed that patients with central nervous system or massive liver involvement were excluded from this study.

Results of survival time studies in both groups of postmenopausal patients with advanced breast cancer were about equal. A possible drawback of tamoxifen as initial therapy for individual patients might be that, in contrast to EE_2, no withdrawal responses have been observed after cessation of tamoxifen.

Regarding side effects of both treatment schedules, it must be stressed that EE_2 therapy was always combined with chlorothiazide. Moreover, patients with congestive cardiac disease were excluded from this treatment because of the known high incidence of fluid retention or congestive cardiac failure during high-dose estrogen treatment (3). In this study, serious fluid retention and cardiac failure were not observed during either EE_2 or tamoxifen therapy.

The most frequent side effect of EE_2 therapy was initial nausea and vomiting, which always ceased within the first week. However, in three patients nausea was accompanied by obstructive liver disease and cholelithiasis. In two of them liver dysfunction persisted until cessation of EE_2 treatment. Both patients had no serious side effects during subsequent tamoxifen therapy. These findings agree with reported data in the literature, which strongly suggest that estrogens (low doses) can cause concentration of bile acids and exacerbations of gallbladder disease in susceptible women (13,14). Tamoxifen also caused nausea and vomiting, although it was much less severe and less frequent than that caused by EE_2. Two patients refused further treatment because of these symptoms: in one, no objective symptoms of toxicity were found, and the other could not be investigated during the period of nausea. Nausea and vomiting due to tamoxifen treatment has been observed in about 10% of the cases reported in the literature (7).

Initial hypercalcemia was observed in both treatment groups but never required cessation of therapy. Kennedy and Nathanson (3) reported only a few cases of estrogen-related severe hypercalcemia. With respect to tamoxifen, severe hypercalcemia is an uncommon side effect (7,15). However, Henningsen and Amberger (16) reported a transient increase of serum calcium levels > 2.65 mmols/liter in 23% of cases. Initial hypercalcemia was not seen in patients with ER-negative tumors.

Initial increase of tumor pain has been observed both in patients receiving EE_2 and in those receiving tamoxifen treatment. This phenomenon has been described for tamoxifen by Plotkin et al (17). Transient exacerbation of tumor pain in relation to estrogen treatment was recognized in an earlier report (18). Initial increase of tumor pain was not seen in patients with ER-negative tumors. Although initial flare-up of pain as well as initial hypercalcemia seems to express hormonal sensitivity of the tumor, they were not always followed by an objective remission in the present study.

Two of 32 patients on tamoxifen treatment developed superficial thrombophlebitis. One of the EE_2-treated patients developed deep venous thrombosis with multiple embolism of the lungs. It is not certain that these kinds of complications are direct side effects of the drugs used, as malignancy itself may be associated with a higher incidence of such complications (19,20). However, in the collected data of Mouridsen et al (7) and in the study of Kennedy and Nathanson (3), thrombophlebitis was mentioned as a possible side effect of treatment with tamoxifen or EE_2. Alterations in thrombocyte counts were not observed during tamoxifen treatment. This contrasts with several reports (4,6, 15,16) describing a transient decrease in platelet counts in up to 10% of the patients receiving tamoxifen. Other side effects of both treatment modalities (table 4) were not severe and are well-documented in the literature (2,3,7).

CONCLUSIONS

EE_2 (combined with chlorothiazide) and tamoxifen were both about equally effective with respect to remission induction and duration in postmenopausal patients with advanced breast cancer. Patients with ER-negative tumors or patients with far-advanced disease did not respond to either EE_2 or tamoxifen. Withdrawal tumor regressions were

observed only in EE_2-treated patients. Survival time studies in both groups were very similar. Side effects in tamoxifen-treated patients appeared to be less serious than those in EE_2-treated patients. It seems reasonable to treat patients with congestive cardiac disease preferentially with tamoxifen, and gallstone disease may be a contraindication for EE_2 therapy.

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Laboratory Parameters as an Alternative to Performance Status in Prognostic Stratification of Patients With Small Cell Lung Cancer[1]

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SUMMARY

Pretreatment performance status (PS) is a major prognostic factor for both treatment response and survival duration in cancer clinical trials. PS is, however, a subjective index that may be markedly influenced by acute self-limited events. The objective of this study was to develop a prognostic index, based on pretreatment laboratory results, that might serve as an alternative to PS in patients with small cell anaplastic lung cancer. Results have been analyzed in 56 newly diagnosed patients with a minimum followup of 10 months and a median followup of 21 months. Patients were divided into high and low groups for each laboratory parameter based on readings either above or below the median value. Patients with high hemoglobin or albumin levels or low serum alpha-1 globulins, gamma globulins, or LDH survived significantly longer than patients with the opposite levels. When these factors were evaluated by multivariate analysis, albumin and hemoglobin were the most influential prognostic factors for survival. After inclusion of these two laboratory parameters, PS was no longer a significant prognosticator of survival. An objective prognostic index based on pretreatment laboratory results appears feasible in patients with small cell lung cancer.

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Performance status (PS) is a major prognostic factor in cancer clinical trials. Asymptomatic, ambulatory patients have higher response rates and longer survival than symptomatic, bedridden patients. PS suffers, however, in that it is a subjective rating that may be markedly influenced by acute events when strictly applied. Thus, an ambulatory, relatively asymptomatic patient with a Zubrod PS of 0 or 1 (1) or a Karnofsky PS of 100–80 (2) may deteriorate to a Zubrod PS of 3 or 4 or a Karnofsky PS of 50–30 because of the occurrence of a pathologic bone fracture, acute electrolyte abnormalities, acute infectious illness, or other acute but self-limited reasons.

A prognostic data base that is less subject to fluctuations induced by acute events would be useful. For this purpose we attempted to determine whether pretreatment biochemical laboratory tests might provide similar prognostic information as PS in patients with small cell cancer of the lung (SCCL). Objectively determined laboratory parameters might then serve as an alternative to PS in both the design and the analysis of clinical trials for such patients.

PATIENTS AND METHODS

Fifty-six consecutive National Cancer Institute-Veterans Administration Medical Oncology Branch patients with newly diagnosed, previously untreated SCCL were studied. There were 42 males

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and 14 females. Seventeen patients had limited disease (confined to one hemithorax with or without bilateral mediastinal and ipsilateral supraclavicular lymph nodes). The remaining patients had disease beyond these limits (extensive disease). Thirty patients had a Zubrod PS of 1, 16 had a PS of 2, and ten had a PS of 3. All patients had normal hematologic, renal, and hepatic functions except when documented hepatic metastases were present.

During the initial staging evaluation, all patients had the following laboratory tests performed: plasma hemoglobin; serum total protein, albumin, and LDH by SMA/12 autoanalyzer; and paper strip electrophoresis determination of alpha-1, alpha-2, beta, and gamma globulins. In addition, each patient's best therapeutic response and survival were recorded.

Patients were treated with cyclic alternating chemotherapy regimens including cyclophosphamide, methotrexate, and CCNU alternating with vincristine, doxorubicin, and procarbazine (3). As part of their primary therapy, 16 patients received VP-16-213 and ifosfamide (3). Twenty-seven patients also received thymosin fraction V (13 received 20 mg/m^2 and 14 received 60 mg/m^2) during the first 6 weeks of treatment (4). In seven patients thoracic radiation therapy, administered after 12 weeks of chemotherapy, was part of the primary treatment program.

Results for each laboratory parameter were divided into "high" or "low" values; the high value was defined as a result equal to or greater than the median value for that variable, while the low value was below the median. This was done to eliminate the possibility that cut-off points were selectively chosen to maximize the difference between groups with respect to survival; such a practice invalidates the usual interpretation attached to a P value.

Laboratory and clinical variables were first considered separately to determine their individual impact on survival. To compare survivorship between two or more groups, univariate analyses were performed using the generalized Kruskal-Wallis test for unequal censoring (5). Tests for trend in survival were performed as described by Peto et al (6). Survival curves were calculated using the method of Kaplan and Meier (7). Based on the Mantel-Haenszel test statistic (8), a multivariate procedure was used to determine the prognostic importance of the factors jointly. This was accomplished by first selecting which laboratory parameter was most highly associated with survival using a chi-square analysis. Remaining laboratory factors were then evaluated relative to the degree to which survival was explained by factors already selected as more important. After all the laboratory parameters significantly associated with survival were determined, the association of clinical variables with survival was evaluated. The Kruskal-Wallis nonparametric one-way analysis of variance, adjusted for ties, was used to evaluate whether the laboratory variable values differed significantly among the three pretreatment PS categories or types of response (9). Contingency tables were analyzed using the Pearson chi-square test, adjusted for continuity where appropriate. All P values in this report correspond to two-sided significance levels.

RESULTS

Pretreatment laboratory parameters were evaluated in 56 newly diagnosed patients with SCCL (table 1). Five pretreatment laboratory parameters and PS were of prognostic importance for survival. Included in the former were plasma hemoglobin, serum albumin, alpha-1 globulin, gamma globulin, and LDH. Patients with a hemoglobin or serum albumin value above the simple median had significantly prolonged survival ($P = 0.026$ and $P < 0.001$, respectively) compared to patients with values below the median. Conversely, patients with serum alpha-1 globulin, gamma globulin, and LDH levels below the median had significantly prolonged survival ($P = 0.005$, $P = 0.006$, and $P = 0.048$, respectively) compared to patients with serum values above the median for these parameters. Survival

TABLE 1.—Pretreatment laboratory parameters

Variable	Median	Range	Normal laboratory values (range)
Hemoglobin (g/100 ml)	12.8	9.6–15.8	14–18 for males, 12–16 for females
Total protein (g/dl)	7.1	4.7–8.6	6.0–8.0
Albumin (g/dl)	3.4	1.7–4.8	3.5–5.0
Alpha-1 globulin (%)	4.9	2.0–12.8	2.4–5.3
Alpha-2 globulin (%)	13.1	4.1–26.7	6.6–13.5
Beta globulin (%)	14.3	8.3–22.2	8.5–14.5
Gamma globulin (%)	21.8	9.9–33.6	10.7–12.0
LDH (milliunits/ml)	233	125.0–990.0	99.0–200.0

curves for each of these parameters are shown in figures 1–5.

Patients with a PS of 1 survived significantly longer than PS 2 ($P = 0.005$) or PS 3 patients ($P = 0.01$) (fig 6), with a significant prognostic trend from PS 1 to PS 2 to PS 3 patients ($P = 0.025$). There was no significant survival difference between PS 2 and PS 3 patients ($P > 0.50$). Patients with limited disease had improved survival relative to those with extensive disease ($P = 0.10$). Patients receiving 60 mg/m² of thymosin survived significantly longer than those receiving 20 mg/m² or no thymosin ($P = 0.032$).

Table 2 shows the median value for each laboratory variable according to PS and demonstrates a statistically significant association between four pretreatment laboratory parameters and pretreatment PS. In particular, serum albumin levels progressively decreased as PS became more impaired, while serum levels of alpha-1 and alpha-2 globulins and LDH progressively increased as PS deteriorated. In addition, there was a tendency for plasma hemoglobin levels to be lower in patients with an unfavorable PS.

The only laboratory variable that was significantly associated with extent of disease was serum LDH ($P = 0.002$). The median LDH value in patients with limited disease was 200 milliunits/ml, and in patients with extensive disease it was 277 milliunits/ml.

Four laboratory variables, hemoglobin, albumin, alpha-1 globulin, and LDH, were associated with best response to therapy. Complete responders to treatment had significantly higher hemoglobin ($P = 0.034$) and albumin ($P = 0.001$) and significantly lower alpha-1 globulin ($P = 0.051$) and LDH ($P = 0.001$) than did partial responders or nonresponders.

To determine which of the above laboratory variables exerted an important and independent effect on survival, 55 patients with recorded values for all parameters were evaluated using the multivariate technique described previously. The factor most highly correlated with survival was serum albumin ($P < 0.001$), with high levels associated with prolonged survival. Plasma hemoglobin was the next most important factor associated with survival ($P = 0.088$), although serum LDH was also important ($P = 0.095$).

Figure 7 illustrates the combined association of serum albumin and plasma hemoglobin with survival. Combinations of these two factors comprised four prognostic categories that were clearly associated with length of survival ($P < 0.0001$), with a significant trend in prognosis ($P < 0.0001$) from the least favorable group (low levels for both factors)

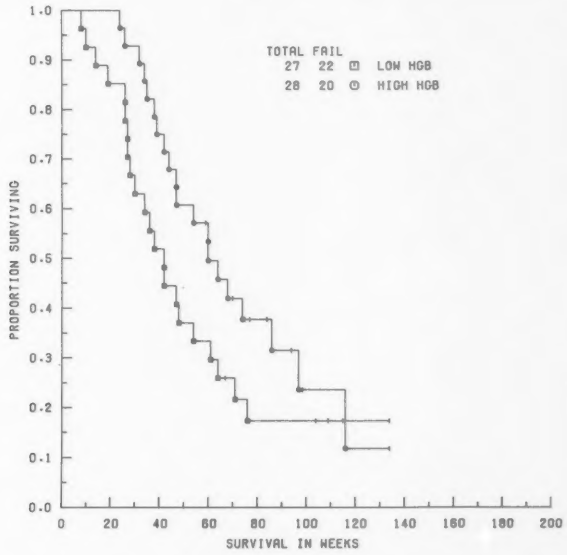

FIGURE 1.—Survival of patients as affected by either a high or low plasma hemoglobin level. Patients with a high hemoglobin survived significantly longer than did those with a low level ($P = 0.026$).

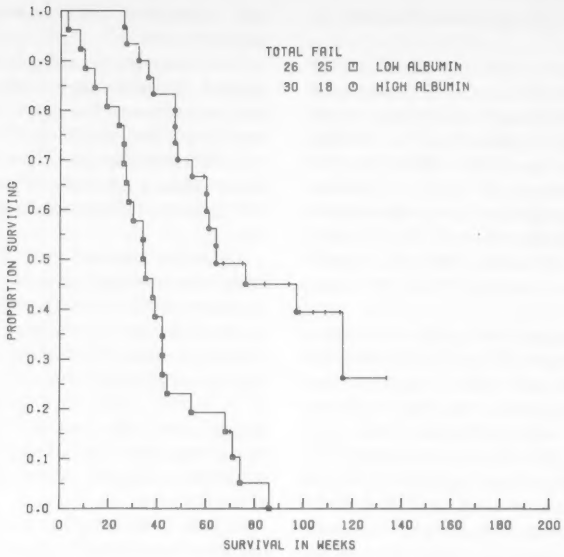

FIGURE 2.—Survival of patients as affected by either a high or low serum albumin level. Patients with high serum albumin survived significantly longer than did those with low values ($P < 0.001$).

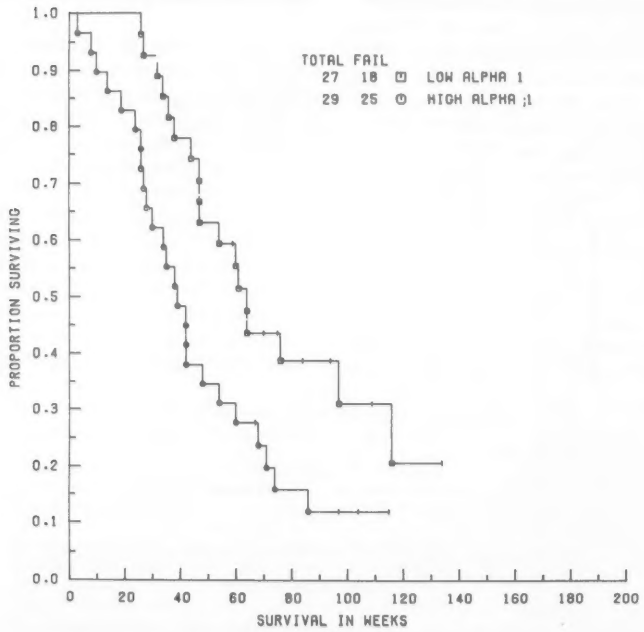

FIGURE 3.—Survival of patients as affected by either a low or high serum alpha-1 globulin level. Patients with low alpha-1 globulins survived significantly longer than did those with high values ($P = 0.005$).

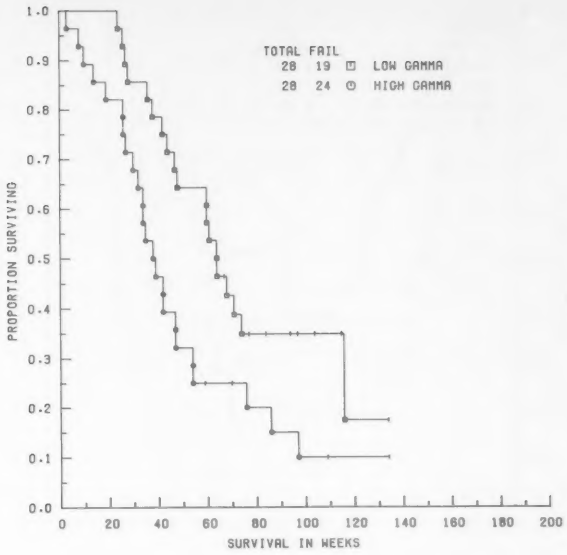

FIGURE 4.—Survival of patients as affected by either a low or high gamma globulin level. Patients with low values survived significantly longer than did those with high values ($P = 0.006$).

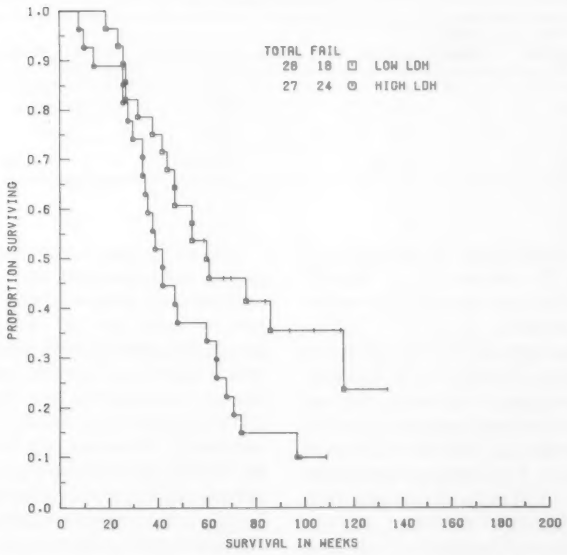

FIGURE 5.—Survival of patients as affected by either a low or high value for serum LDH. Patients with low values survived significantly longer than did those with high values ($P = 0.048$).

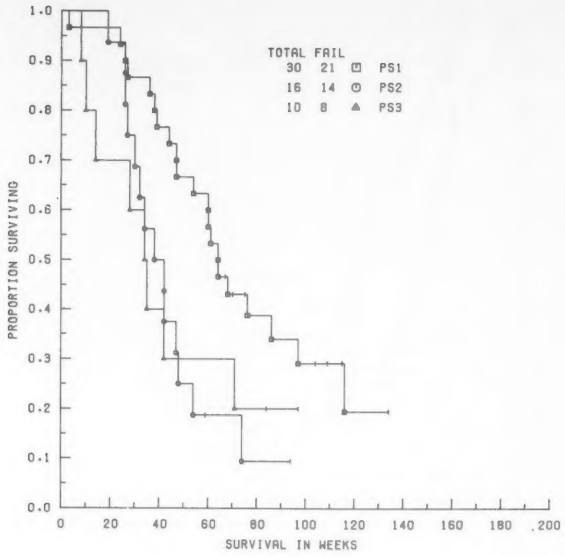

FIGURE 6.—Prognostic influence of PS on survival. Patients with a PS of 1 survived significantly longer than did those with a PS of 2 or 3.

TABLE 2.—Laboratory variables in relation to pretreatment PS

	Median value for—				
PS	Albumin (g/dl)	Alpha-1 globulin (%)	Alpha-2 globulin (%)	LDH (milliunits/ml)	Hemoglobin (g/100 ml)
1	3.5	4.3	12.1	220	13.1
2	3.3	4.9	13.7	231.5	12.6
3	2.9	6.4	15.1	470	11.9
P value	0.012	0.027	0.028	0.001	0.088

to the most favorable group (high levels for both factors). Furthermore, PS was no longer significantly related to survival after accounting for serum albumin and plasma hemoglobin levels ($P = 0.75$).

To evaluate further the interrelationship of serum albumin, LDH, and plasma hemoglobin levels with extent of disease, the number of extensive disease sites was first determined for each patient. No sites of extensive disease (denoted 0) indicated that the patient had limited disease. A refined categorization of extensive disease was defined as either one, two, or three or more sites of extensive disease, providing a more sensitive measure of the patient's disease extent. The joint relationship of these three laboratory variables with this refined classification of disease extent is illustrated in table 3.

Several types of analyses including the log-linear model technique (18) were applied to these data as well as to a reduced table in which patients with two or more sites of extensive disease were combined. No strong evidence was present to suggest that a significant association exists among these laboratory parameters and disease extent, except for LDH as stated previously. This conclusion must be tempered, however, by the fact that only a small number of observations per category were present in table 3. When only albumin and hemoglobin were jointly evaluated in regard to their association with disease extent, there was a degree of association between the two variables and disease extent (table 4). Patients with the more prognostically favorable levels of serum albumin and plasma hemoglobin

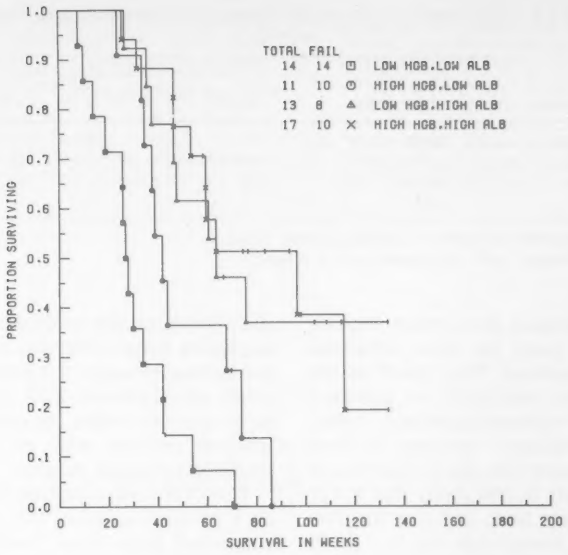

FIGURE 7.—Survival as related to pretreatment laboratory values for serum albumin and plasma hemoglobin. Patients with high values for each of these parameters had the most favorable prognosis, and those with low values had the worst prognosis.

TABLE 3.—Joint relation of albumin, plasma hemoglobin, and LDH levels with disease extent*

			No. of sites of extensive disease†			
Albumin	Hemoglobin	LDH	0	1	2	≥ 3
High	High	High	1	2	1	3
		Low	6	3	1	0
	Low	High	2	1	2	0
		Low	6	1	1	0
Low	High	High	2	2	0	2
		Low	4	0	1	0
	Low	High	0	4	3	2
		Low	2	1	1	1

**See* text for definition of high and low values.
†Values = No. of patients in each category.

tended to have less extensive disease than did those with less favorable levels.

DISCUSSION

The purpose of this study was to determine the usefulness of pretreatment laboratory parameters as alternative prognostic factors to PS with regard to response to therapy and duration of survival for patients with SCCL. Several laboratory determinations had significant prognostic importance. Patients who had plasma hemoglobin or serum albumin levels above the median value for the study population or who had serum alpha-1 globulins or LDH levels below the median values had significantly prolonged survival relative to patients on the opposite side of the median. Alpha-2 globulin levels were less significantly correlated with survival ($P = 0.12$). Serum albumin, alpha-1 globulin, alpha-2 globulin, and LDH correlated significantly with PS, with serum albumin progressively increasing and the other parameters progressively decreasing with improved PS.

An analysis in which these factors were consid-

TABLE 4.—Joint relation of albumin and plasma hemoglobin levels with disease extent*

Albumin	Hemoglobin	No. of sites of extensive disease†			
		0	1	2	≥ 3
High	High	7	5	2	3
	Low	8	2	3	0
Low	High	6	2	1	2
	Low	2	5	4	3

*See text for definition of high and low values.
†Values = No. of patients in each category.

ered simultaneously indicated that serum albumin and plasma hemoglobin were the most influential prognostic factors for survival. The cut-off values used in the analysis and selected in an unbiased manner correspond well with our laboratory limits, thereby lending some biologic relevance to these cut-off points. Table 1 shows that the median serum albumin value for patients in this study was 3.4 g/dl (low normal laboratory limit is 3.5 g/dl). The median value for plasma hemoglobin was 12.8 g/100 ml (low normal laboratory limit is 14 g/100 ml for males and 12 g/100 ml for females). Similar conclusions are obtained if results are recalculated utilizing the low laboratory limit for normal serum albumin and plasma hemoglobin.

Several investigators have correlated declining serum albumin levels (10,11) and declining plasma hemoglobin levels (12) with advancing malignancy. Similarly progressive increases in serum alpha-1 and alpha-2 globulin levels, in advanced cancer patients, reflect increased hepatic synthesis of acute-phase glycoproteins (13). These latter proteins include alpha-1 antitrypsin, alpha-1 acid glycoprotein, ceruloplasmin, and haptoglobin (14). Elevations of serum alpha-1 and alpha-2 globulins are not specific for malignancy but can occur in a wide variety of inflammatory diseases as well as with trauma. The reason for increased hepatic synthesis of acute-phase reactants following disease or injury is uncertain, although one postulate is that these proteins are required to suppress host immune response to auto-antigens (15).

Serum levels of LDH were found to correlate with disease extent. Other malignant diseases where a similar correlation has been made include American Burkitt's lymphoma (16) and Ewing's sarcoma (17).

Based on pretreatment laboratory tests, serum albumin and plasma hemoglobin concentrations provided an excellent prognostic discriminant for survival. Comparison of figures 6 and 7 suggests that the four groups defined by these two factors can serve as an alternative to PS as a prognosticator for survival. The group of patients with SCCL with the most favorable prognosis would have normal or increased serum albumin and plasma hemoglobin levels (ie, albumin ≥ 3.4 g/dl and hemoglogin ≥ 12.8 g/100 ml). Patients with a poor prognosis would have opposite values for each of these parameters, whereas patients with an intermediate prognosis would have mixed results.

The utility of objective biochemical parameters as a prognostic index for patients with SCCL is suggested from these results. Additional evaluations of these factors in larger groups of patients with SCCL seem warranted.

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Treatment of Advanced Adenocarcinoma of the Lung With Ftorafur, Doxorubicin, Cyclophosphamide, and Cisplatin (FACP) and Intensive Iv Hyperalimentation[1,2]

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SUMMARY

Sixty-five patients with extensive adenocarcinoma of the lung were treated with ftorafur, doxorubicin, cyclophosphamide, and cisplatin (FACP). Twenty-four received no iv hyperalimentation (IVH) (FACP alone); 19 received simultaneous IVH (S-IVH) beginning on Day 1 and continuing through the first FACP course (median, 25 days), and 22 received prechemotherapy IVH (P-IVH) beginning 10 days prior to FACP and continuing through the first course (median, 35 days). Groups were comparable except for a higher proportion of patients with less pretreatment weight loss in the group that received FACP alone. Of 48 patients with measurable disease, responses ≥ 50% were seen in 15 (four complete and 11 partial remissions): seven of 18 patients (39%) who received FACP alone, two of 13 (15%) who were given S-IVH, and six of 17 (35%) who received P-IVH. Of the 15 responding patients, 93% were fully active and ambulatory (class A), 87% had < 6% pretreatment weight loss, 60% were above the group median for daily nitrogen intake, and 70% were above the group median for daily caloric intake. The 40-week median survival for all patients receiving FACP alone appeared better than the median of 28 weeks for patients receiving P-IVH ($P = 0.009$) and 21.5 weeks for patients treated with S-IVH ($P = 0.09$). However, when the data were corrected for imbalances of prognostic factors, there were no differences in survival among the three treatment groups. Survival was prolonged in patients with < 6% pretreatment weight loss ($P = 0.03$), in responding patients ($P = 0.01$), and in patients with stable disease ($P = 0.02$). Five of 16 responding patients remained alive for ≥ 18 months. Protection from chemotherapy-induced gastrointestinal and hematologic toxic effects by IVH was minimal. Nutritional parameters have prognostic significance in patients with advanced adenocarcinoma of the lung. The routine use of IVH as an adjunct to chemotherapy in patients with adenocarcinoma of the lung does not appear to be justifiable.

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The prognostically adverse effects of pretreatment protein-calorie malnutrition, which are reflected in such parameters as weight loss and poor performance status in patients with lung cancer and other malignant diseases, have been recognized (1–4). Furthermore, cancer therapy frequently adds to the severity of the malnourished state (5–7). Recently, a report from our institution demonstrated protection from chemotherapy-induced neutropenia in patients with non-oat cell lung cancer who received nutritional supplementation via hyperalimentation while being treated with combination chemoimmunotherapy (8).

The significant activity of doxorubicin alone and in combination with cyclophosphamide and cisplatin in patients with bronchogenic carcinoma has been demonstrated (9–12). In addition, doxorubicin combined with 5-FU and methotrexate produced an encouraging response rate of 45% in 11 patients with adenocarcinoma of the lung (13). Ftorafur has a spectrum of antineoplastic activity similar to that of 5-FU, though it is significantly less myelosuppressive (14–16). A previous study at our institution which used ftorafur, doxorubicin, and cisplatin in the treatment of patients with adenocarcinoma and large cell anaplastic carcinoma of the lung demonstrated responses in ten of 24 patients (42%) with measurable disease (17).

Considering the above information, a study was designed to investigate the impact of intensive nutritional supplementation in patients with extensive adenocarcinoma of the lung who were receiving combination chemotherapy with ftorafur, doxorubicin, cyclophosphamide, and cisplatin (FACP).

MATERIALS AND METHODS

Patients with inoperable, histologically documented adenocarcinoma of the lung who had not previously been treated with any of the study drugs were entered into the study. In the initial part of the study, patients were randomized to receive FACP without nutritional supplementation, designated as the FACP group, or FACP plus iv hyperalimentation (IVH). In the latter group, both the FACP and IVH began on Day 1 of the first course. The IVH was continued throughout the first course (median, 25 days). This group was designated as the simultaneous IVH (S-IVH) group. After 43 patients were entered into the study (24 FACP and 19 S-IVH), a preliminary analysis revealed no protection from myelosuppression, which was contrary to our previous study (8). The chemotherapeutic agents used in the two studies were different. However, the only difference in the treatment schedules was the administration of IVH at least 10 days prior to chemotherapy in the earlier study. Therefore, a third group of 22 patients was added in a nonrandomized fashion, in which IVH was begun 10 days prior to the onset of FACP chemotherapy and was continued throughout the remainder of the first course (median, 35 days). This was designated as the pretreatment IVH (P-IVH) group. Otherwise, no other differences in treatment existed among the groups.

The chemotherapy was administered at 21-day intervals. Ftorafur (3 g/m^2 in 200 ml of 5% dextrose solution) was given over a 4-hour period on Days 1 and 4. Doxorubicin (60 mg/m^2 in 50 ml of 5% dextrose solution) and cyclophosphamide (500 mg/m^2 in 200 ml of 5% dextrose solution) were each given over 15 minutes on Day 1. Cisplatin (15 mg/m^2 in 500 ml of 5% dextrose solution) was given over a 4-hour period on Days 1–4, with additional emphasis on hydration during and after the treatment period.

Two types of central venous catheters were used to administer the IVH solution. In 33 patients, a 16-gauge, 20-inch silicone elastomer catheter (Intrasil, Vicra Corp) with an internal volume of 0.4 ml was introduced percutaneously in the basilic or cephalic vein at the antecubital fossa and advanced to the superior vena cava. In five patients, a percutaneous subclavian vein approach was used with a 16-gauge, 9-inch silicone elastomer catheter (Centrasil, Vicra Corp) with an internal volume of 0.2 ml. Strict aseptic technique was used for catheter insertion and during dressing changes three times weekly. The IVH fluid consisted of a standard solution of 500 ml of 50% glucose and 500 ml of 8.5% amino acid solution, with electrolytes, vitamins, and minerals added daily. We attempted to deliver a minimum of 3000 calories iv per day, in addition to oral nutrition ad libitum.

A thorough evaluation of disease status was made in each patient before each course for the first three courses and then at 6-week intervals, in an attempt to document and follow all disease. A complete remission (CR) was defined as the disappearance of all evidence of disease. A partial remission (PR) was defined as a $\geq$ 50% decrease in the sum of the products of two perpendicular diameters of all measurable lesions, without an increase in any existing lesion. To be called a response, a CR or PR had to be observed. A PR in patients with histologically documented but nonmeasurable disease consisted of radiologic tumor regression of $\geq$ 50% as determined by two independent examiners. A less than partial remission was defined as a decrease between 25% and 50%. Stable disease was defined as a change of $\leq$ 25%, and progressive disease was defined as a $\geq$ 25% increase in any measurable lesion. If a new lesion developed, regardless of other re-

TABLE 1.—Characteristics of patients treated with FACP, S-IVH, or P-IVH

Characteristics*	FACP	S-IVH	P-IVH
Registered	24	19	22
Inevaluable (early death)	1	2	1
Evaluable	23	17	21
Sex			
Male	16	10	12
Female	7	7	9
Age in yrs			
Median	61.5	60	54
Range	43–69	37–76	38–73
Performance status			
Class A	19	9	12
Class B	4	5	7
Class C	0	3	2
Pretreatment weight loss			
< 6%	13	8	13
6%–12%	10	3	6
> 12%	0	6	2
Extent of disease			
Extensive	19	13	20
Limited	4	4	1
Evaluability of disease			
Measurable	18	13	17
Evaluable	5	4	4

*Unless specified otherwise, values = No. of patients.

sponse, the patient was considered to have disease progression.

Immunologic evaluation was performed by the use of delayed hypersensitivity skin tests to recall antigens (dermatophyte, *Candida*, mumps, Varidase, and purified protein derivative of intermediate strength) as described by Hersh et al (18). Each patient was placed into performance status category A, B, or C. Class A patients were those who were ambulatory and fully active. Class B patients required nursing assistance or the equivalent or were bedridden for less than half of a normal day. Class C patients were bedridden for more than half of a normal day.

Thorough nutritional evaluation according to the methods described by Blackburn et al (19) was made before and during treatment. This consisted of body weight changes, nitrogen balance, creatinine-height index, arm muscle circumference, triceps skin fold measurements, and percent recommended daily allowance (RDA) of kilocalories and nitrogen intakes. In addition, cbcs and platelet counts were performed three times per week, and overall chemistry survey and urinalysis were performed weekly for the first four courses.

Response rates and incidence of toxic effects were compared by chi-square tests. Measures of myelosuppression were compared using a Wilcoxon test, and Gehan's modification of the Wilcoxon test (20) was used to test differences in survival distributions. Survival curves were plotted using the method of Kaplan and Meier (21).

RESULTS

Patient Characteristics

Table 1 shows the distribution of patient characteristics. A total of 65 patients were entered in the study. The groups were comparable except for a preponderance of patients with less pretreatment weight loss ($P > 0.02$) and better performance status ($P > 0.09$) among those randomized to receive chemotherapy without nutritional supplementation (FACP group). The median age of patients in the P-IVH group was slightly lower.

Response

Sixty-one of the 65 patients entered were evaluable for response. The remaining four patients were considered to have had early deaths, defined as death within the first course of treatment and not as a sequela of the therapy. One patient who

TABLE 2.—Response related to treatment schedule and to the presence of measurable or evaluable disease*

Response	FACP		S-IVH		P-IVH		Total	
	Meas	Eval	Meas	Eval	Meas	Eval	Meas	Eval
CR	2	—	—	—	2	—	4	—
PR	5	—	2	—	4	1	11	1
CR + PR	7(39%)	—	2(15%)	—	6(35%)	1	15(31%)	1
< PR	3	—	2	—	—	—	5	—
Stable disease	5	3	5	3	5	1	15	7
Disease progression	3	2	4	1	6	2	13	5
Total	18	5	13	4	17	4	48	13

*Meas = measurable, Eval = evaluable. Values = No. of patients.

received FACP alone died on Day 9 of treatment, from aspiration pneumonia and *Clostridium* sepsis. Of the two early deaths in the S-IVH group, one patient died on Day 9 of therapy from gram-negative sepsis, and the other died from a massive pulmonary embolus (not catheter related) on Day 11. The remaining early death occurred in the P-IVH group: the patient had a fatal cerebrovascular accident (unrelated to tumor) on Day 4 of the therapy. In a patient who was considered to have disease progression, tumor growth appeared to be faster during IVH therapy, although no objective documentation of this could be made. A similar observation was made in one other patient in the P-IVH group who later achieved CR.

Table 2 shows the response rates of the three treatment groups relative to the presence of measurable or evaluable disease. Overall, 15 of 48 (31%) patients with measurable disease responded with CR or PR. An additional five patients responded with remissions less than partial, for a total of 20 (42%) objective tumor responses. There was only one response (PR, P-IVH) among 13 patients with evaluable disease. Among patients with measurable disease, the CR plus PR rates were 39% for FACP, 15% for S-IVH, and 35% for P-IVH, although these differences did not reach statistical significance ($P \geq 0.3$). The combined response (CR plus PR) for patients with measurable and evaluable disease was 30% for FACP, 12% for S-IVH, and 33% for P-IVH. If early deaths are included in the denominators, the combined response for patients with measurable and evaluable disease was 29% for FACP, 11% for S-IVH, and 32% for P-IVH.

The relationship between response, treatment schedule, and prognostic factors is seen in table 3. Overall, responses were more common in patients with class A performance status (14 of 40, 35%) than in those with class B (two of 16, 13%) or class C (none of five). Similarly, responses were more common among patients with < 6% pretreatment weight loss (13 of 34, 38%) than patients with 6%–12% weight loss (three of 19, 16%) or those with > 12% weight loss (none of eight). Responses were similar in patients with limited (three of nine, 33%) and extensive (13 of 52, 25%) disease. The difference in response in favor of patients with class A performance status and < 6% pretreatment weight loss did

TABLE 3.—Response related to prognostic factors*

	FACP		S-IVH		P-IVH		Total	
	Enr	Resp	Enr	Resp	Enr	Resp	Enr	Resp
Total	23	7	17	2	21	7	61	16
Performance status								
Class A	19	7	9	2	12	5	40	14
Class B	4	0	5	0	7	2	16	2
Class C	0	0	3	0	2	0	5	0
Pretreatment weight loss								
< 6%	13	6	8	1	13	6	34	13
6%–12%	10	1	3	1	6	1	19	3
> 12%	0	0	6	0	2	0	8	0
Extent of disease								
Limited	4	3	4	0	1	0	9	3
Extensive	19	4	13	2	20	7	52	13

*Enr = enrolled, Resp = responding, Values = No. of patients.

TABLE 4.—Response relative to nutritional factors*

Parameters	Median	No. of patients below median		No. of patients above median	
		Enr	Resp	Enr	Resp
Arm muscle circumferences†	97%	19	4 (21%)	20	8 (40%)
Triceps skin fold†	75%	19	4 (21%)	20	8 (40%)
Creatinine-height index‡	77%	16	3 (19%)	17	8 (47%)
Nitrogen balance	−1.17	11	2 (18%)	11	4 (36%)
Mean daily kcal intake	2770	21	3 (14%)	22	7 (32%)
% RDA of kcal	118	21	4 (19%)	22	6 (27%)
Mean daily N intake (g)	15.4	21	4 (19%)	22	6 (27%)
% RDA of N	105	21	4 (19%)	22	6 (27%)
Post-treatment weight change	+3%	16	3 (19%)	15	6 (40%)

*Enr = enrolled, Resp = responding.
†Expressed as % of Jelliffe standard for normal subjects.
‡Expressed as % of Bistrian standard for normal subjects.

not achieve statistical significance ($P \geq 0.09$). The administration of IVH, whether S-IVH or P-IVH, did not improve the response rate to FACP alone in patients with measurable disease and favorable prognostic factors (class A performance status or pretreatment weight loss $< 6\%$) or unfavorable prognostic factors (class B and C performance status or pretreatment weight loss of $\geq 6\%$). The lower response rate for patients receiving S-IVH did not reach statistical significance. Of 36 patients with measurable lesions who underwent pretreatment skin tests, responses appeared more common among patients with positive reactions (11 of 26, 42%) than with negative reactions (two of ten, 20%) ($P = 0.3$), although there were no differences among treatment schedules.

Table 4 shows response rates relative to nutritional factors. The number of patients varies among the nutritional parameter groups because of failure to keep the appropriate data by patient or because of technical difficulties in collecting and recording such data (especially true for nitrogen balance calculations). For each nutritional parameter, the median was established for the entire group, regardless of treatment, and patients were categorized as above or below the median. Although statistical significance was not achieved in any nutritional category, a definite trend toward a response advantage was seen in all categories that were above the median for the group.

Survival

The survival duration for all patients entered in the study is shown in figure 1. The apparent survival advantage for the FACP group over the S-IVH ($P = 0.009$) and the P-IVH ($P = 0.09$) groups was related to the large proportion of patients with $> 12\%$ pretreatment weight loss in the S-IVH group. The exclusion of all such patients from the survival calculations minimized the differences ($P > 0.07$), although it still favored the patients who received FACP alone. Response to therapy was associated with improved survival time as shown in figure 2. The median survival of patients who achieved CR or PR exceeded 1 year; five of 16 patients lived for ≥ 18 months. The survival differences between patients who achieved tumor remission and patients whose disease became stable or progressed were statistically significant ($P > 0.01$). The administration of IVH did not influence the survival of patients whose disease became stable or progressed. However, the survival of responding patients receiving FACP alone appeared to be slightly longer ($P = 0.15$–0.04). Patients with class A performance status and those with $< 6\%$ pretreatment weight loss had a survival advantage ($P = 0.03$).

Toxicity

Myelosuppression was the most frequently observed severe toxic effect of chemotherapy, with neutropenia being the most significant. In an attempt to determine the precise impact of nutrition and nutritional supplementation on drug-induced myelosuppression, patients were selected in each treatment category for comparability. Prior irradiation was considered an unpredictable variable, and patients who had previously been treated with radiation were excluded. Patients were also matched according to pretreatment bone marrow cellularity. The percent of body weight change in each group showed a striking difference, with patients who received FACP alone losing a median of 2.5% of pretreatment body weight during the first course and

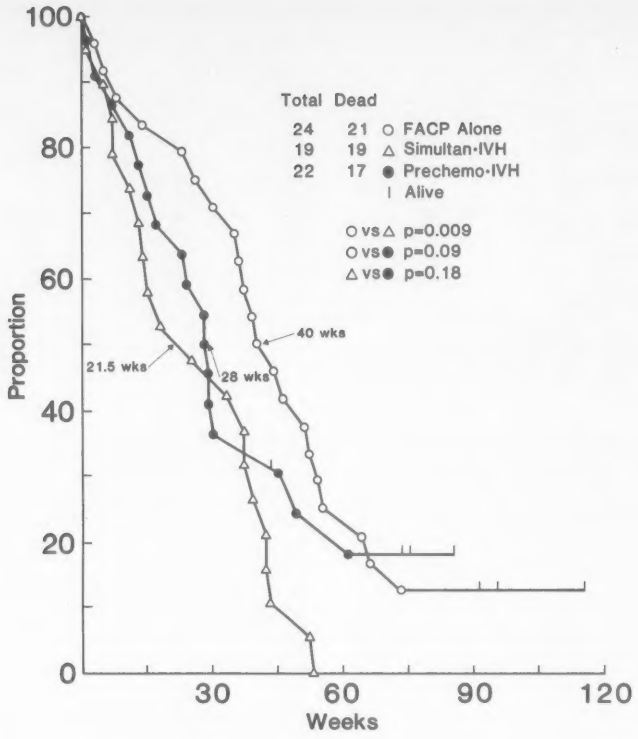

FIGURE 1.—Survival relative to treatment schedule.

patients in the S-IVH and P-IVH groups gaining 4% and 9%, respectively. These relationships are shown in table 5. No significant protection from leukopenia or neutropenia was seen in any treatment category during the first two courses of chemotherapy ($P > 0.05$). However, the administration of P-IVH resulted in protection from thrombocytopenia only during the first course of chemotherapy when compared to S-IVH ($P = 0.055$) but not when compared to FACP ($P = 0.29$). Comparison between FACP and S-IVH showed no significant difference ($P = 0.48$).

Other side effects observed during the first course and the remaining courses of therapy within each treatment schedule are shown in table 6. Nausea and vomiting were universal side effects and were severe, lasting > 24 hours after chemotherapy in 43% of patients who received FACP and in 35% and 29% of those who received S-IVH and P-IVH, respectively ($P \geq 0.6$). Conversely, the frequency of mild nausea and vomiting was greater in the P-IVH (38%) and the S-IVH (24%) groups than in controls (10%) ($P > 0.04$). There were 27 (8%) episodes of proven infection during 334 treatment courses. The most common infection was pneumonia (11 episodes) followed by urinary tract infection (UTI) (seven), septicemia (five), and soft tissue infection (four). Fifteen infections (50%) occurred during the first course of therapy: two in the FACP group, six in the S-IVH group, and seven in the P-IVH group ($P \geq 0.1$). Of these, there were seven episodes of pneumonia (FACP group, one; S-IVH group, three; and P-IVH group, three), five episodes of UTI (S-IVH group, one; and P-IVH group, four), two episodes of septicemia (FACP group, one; and S-IVH group, one), and one case of cellulitis (S-IVH group). Eight infections were caused by gram-negative bacilli, three by gram-positive cocci,

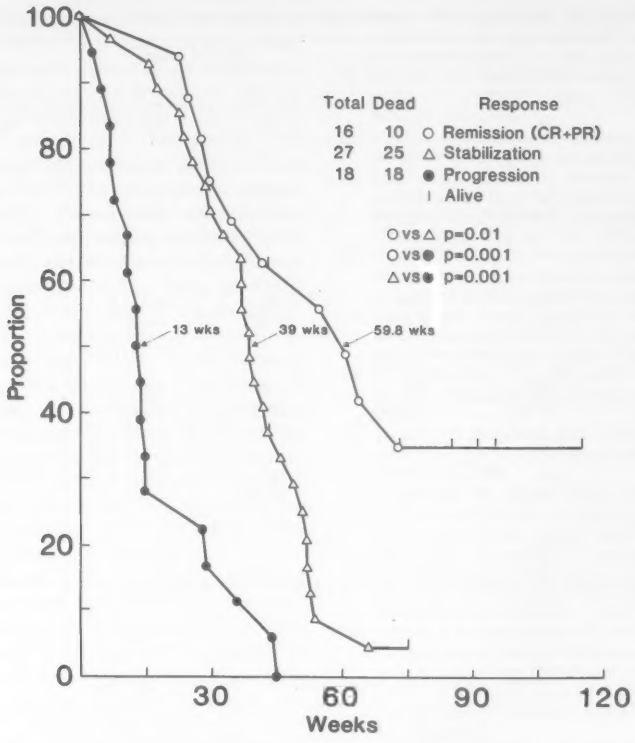

FIGURE 2.—FACP chemotherapy with and without IVH: survival relative to response.

and four by unidentified organisms. In no case was an infection thought to be a result of hyperalimentation or catheter contamination. Hyperglycemia was frequently seen in patients who received IVH but was usually easily controlled with the addition of insulin to the IVH solution and was generally transient, occurring usually only during the first 10 days of IVH. Venous thrombosis necessitating catheter removal occurred in seven patients receiving IVH and was thought to result in a nonfatal pulmonary embolus in one patient. Other toxic effects were acceptable and consistent with similar regimens.

DISCUSSION

Compared to intensive chemotherapy alone, the addition of nutritional supplementation in the form of IVH did not improve the response or survival rate of patients with advanced inoperable adenocarcinoma of the lung. On the contrary, the response rate and survival duration of the IVH-treated patients was somewhat inferior to that of patients treated with chemotherapy alone. However, these differences could be explained, in part, on the basis of an uneven distribution of some prognostic factors in the study groups. The overall 31% response rate in patients with measurable disease and the median survival durations of > 1 year for responding patients and 10 months for patients who achieved disease stabilization are consistent with or improvements over most other recently reported similar studies of multiple-agent chemotherapy in non-oat cell bronchogenic carcinoma (21–27). In addition, there exists the potential for long-term survival for five of 16 responding patients who remained alive for > 18 months.

The administration of IVH, whether simultaneous with or preceding chemotherapy was not associated with significant protection from chemo-

TABLE 5.—Myelosuppressive toxicity during the first two courses of chemotherapy

	FACP course		S-IVH course		P-IVH course	
	1	2	1	2	1	2
Total No. of patients	19	16	17	16	19	12
Wbc count (cells × 10^3/mm^3)						
Median	1.5	1.95	1.7	2.1	1.95	2.6
Range	0.8–4.6	0.5–4.8	0.7–4.3	0.5–7.8	0.1–3.	0.6–5.5
Polymorphonuclear leukocyte count (cells × 10^3/mm^3)						
Median	0.5	0.85	0.2	0.8	0.5	0.8
Range	0–1.8	0–2.3	0–2.1	0–4.6	0–1.2	0–3.5
Platelet count (cells × 10^3/mm^3)						
Median	183	181	140	105	214	150
Range	50–415	23–490	24–267	25–497	24–347	37–275
% pretreatment bone marrow cellularity						
Median	25		30		30	
Range	15–45		20–40		5–55	
% body weight change						
Median	–2.5		+4		+9	
Range	–11–1		–1–12		–7–14	

TABLE 6.—Other toxic effects relative to treatment schedule*

	FACP course		S-IVH course		P-IVH course	
	1	Others	1	Others	1	Others
No. of courses evaluable for toxicity	24	117	19	62	22	90
Nausea and/or vomiting	22	117†	18	62†	20	90†
Severe (> 24 hrs)	9		6		6	
Moderate (12–24 hrs)	11		7		6	
Mild (< 12 hrs)	2		5		8	
Infections						
Proven	2	4	6	4	7	4
Fever of undetermined origin	3	2	3	2	1	2
Hyperglycemia	0	0	9	0	6	0
Venous thrombosis	0	0	1	0	6	0
Mucositis	2	3	2	2	0	0
Hypotension	0	2	0	1	1	1
Hypertension	0	1	0	0	0	0
Azotemia	0	4	0	2	0	3
Electrolyte imbalance	0	0	3	0	1	0
Congestive heart failure	1	0	0	1	0	0
Pulmonary embolus	0	0	1	0	1	0
Cardiac toxicity	0	2	0	0	0	0
Confusion and/or memory loss	1	0	2	1	0	0
Neurotoxicity	0	1	0	1	0	0

*Unless specified otherwise, values = No. of episodes.
†Inadequate information to judge severity.

therapy-induced gastrointestinal or myelosuppressive toxic effects. Only the P-IVH group appeared to have had some benefit, as evidenced by a lower incidence of severe vomiting during chemotherapy (29%) in relation to the S-IVH (35%) and the FACP (43%) groups ($P > 0.6$) and by protection from thrombocytopenia when compared to the S-IVH group ($P = 0.05$) but not when compared to controls ($P = 0.29$). These observations vary from those of a pilot study previously reported from this institution, in which patients received different chemotherapy (8), and they differ from an early analysis of the present study (28), in which significant protection from myelosuppression was observed in patients receiving P-IVH. These discrepancies could be accounted for by the differences in the chemotherapy administered, as well as by the limited number of these observations. A disturbing finding of this study was the slight, though not significant, increase of infectious complications in the IVH-treated patients, which did not appear to be related to the contamination of iv catheters. The role of IVH in these events is unknown.

The administration of IVH as an adjunct to antineoplastic chemotherapy needs further exploration. Possible hints of protection from toxic effects of chemotherapy need to be clarified in relation to the type of treatment to be administered, the timing of the IVH relative to that of chemotherapy, and the composition and duration of the IVH. With malignant lesions that show an increased dose-response relationship, the ability to provide mechanisms that protect against treatment toxicity may afford improved therapy and thus improved response and, ultimately, improved survival rates. From the results of this study, however, it appears that the routine use of IVH in patients with adenocarcinoma of the lung is not justified.

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Combination Chemotherapy Salvage of Heavily Pretreated Patients With Hodgkin's Disease: An Analysis of Prognostic Factors in Two Chemotherapy Trials and the Literature[1,2,3]

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SUMMARY

The results of two intensive combination chemotherapy trials for heavily pretreated patients with Hodgkin's disease were markedly different. Only one of 27 patients treated with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) achieved a complete remission (CR), while a CR was attained by 50% of the patients (nine of 18) treated with ABVD alternating with mechlorethamine, vincristine, procarbazine, and prednisone (MOPP/ABVD). There was a significantly longer survival for patients on MOPP/ABVD. Characteristics of patients in the two trials which might have at least partially explained these differing results were identified. The patients treated with ABVD all had prior histories of multiple relapses or continuous disease activity, while the majority of responding patients treated with MOPP/ABVD had few prior relapses and long disease-free intervals immediately prior to treatment. In the MOPP/ABVD trial, a trend toward a higher CR rate was found for patients with relapses confined to lymph nodes, compared with patients who had extranodal disease, although the difference lacked statistical significance. The characteristics of the patient population undergoing treatment must be considered in the interpretation of the results of chemotherapy salvage regimens for Hodgkin's disease.

[Cancer Treat Rep 65:207–211, 1981]

The prognosis of patients with advanced Hodgkin's disease has improved significantly with the use of intensive combination chemotherapy (1). In untreated patients, the use of mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) alternating with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) combined with low-dose radiotherapy (RT) produced a 100% response rate, with 80% of patients in complete remission (CR) and 20% in partial remission (PR) at 24 months median followup time from initiation of treatment (2,3). Re-treatment with combination chemotherapy of patients who relapse after extended field RT has had similar success. Among patients re-treated with the MOPP/ABVD program after relapse following extended field RT and/or small amounts of single-agent chemotherapy, 65% achieved a CR and 24% had a PR (3).

Salvage of patients with Hodgkin's disease who relapsed after extensive chemotherapy alone or

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chemotherapy and RT has been more problematic. Recently, Santoro and Bonadonna (4) reported a 62% CR rate with ABVD in patients resistant to MOPP. Approximately two thirds of the patients achieving a CR remained disease-free at 36 months from initiation of re-treatment (4). Our results (5) and those of others (6,7) were less successful with ABVD in heavily pretreated patients with Hodgkin's disease. To our surprise, our results with MOPP/ABVD in heavily pretreated patients were superior to our experience with ABVD alone. It seemed possible that this discrepancy and those reported for salvage chemotherapy combinations in the literature might be due to differences in patient characteristics in the various trials. If patient characteristics were found to be an important factor in predicting success of salvage treatment with combination chemotherapy, it might be possible to determine which patients would be most likely to benefit from this approach.

PATIENTS AND METHODS

Twenty-seven heavily pretreated patients with Hodgkin's disease were re-treated between November 1974 and July 1977 with ABVD, and 18 heavily pretreated patients were re-treated with MOPP/ABVD between March 1975 and March 1978. All patients had relapsed after MOPP or failed at least one course of MOPP or variants of MOPP. Most patients had multiple relapses after RT as well as combination chemotherapy, and many had received multiple courses of chemotherapy. Patients treated with ABVD received doxorubicin (25 mg/m^2 iv), vinblastine (6 mg/m^2 iv), and dacarbazine (250 mg/m^2 iv) on Days 1 and 15 and bleomycin (2 mg/day sc) on Days 4–12 and 17–26. All patients were treated with a maximum of six monthly cycles. ABVD was discontinued if the patient's disease clearly progressed during treatment. Patients treated with MOPP/ABVD received alternating monthly courses of ABVD and MOPP (2) for eight cycles. The latter program consisted of mechlorethamine (HN2) (6 mg/m^2 iv; maximum, 10 mg) and vincristine (1.4 mg/m^2 iv; maximum, 2 mg) on Days 1 and 8, procarbazine (100 mg/m^2 orally; maximum, 150 mg) on Days 1–14, and prednisone (40 mg/m^2 orally) on Days 1–14 in cycles 1 and 7. When nausea and vomiting with HN2 were severe, it was permissible to substitute cyclophosphamide (650 mg/m^2 iv) or thioTEPA (15–18 mg/m^2 iv) on Days 1 and 8 for HN2. MOPP/ABVD was discontinued in patients who had disease progression during treatment. No additional RT was administered to any patients. The toxicity of these two regimens was comparable and has been previously reported in detail (2,3,5).

CR was defined as the disappearance of all measurable disease parameters during treatment for at least 1 month. PR was defined as the diminution in size of all measurable disease parameters by > 50% for at least 1 month. Treatment failures were defined as the patients achieving less than a PR.

Survival curves were calculated by the Kaplan-Meier product limit method with survival times measured in months from start of therapy (8). For the comparison of the survival patterns of two groups, the log-rank test was applied (9). This test controlled for other factors thought to be associated with prognosis and was used to detect differences in survival times of the groups. The Fisher exact test was used to compare two proportions, and the chi-square test was used to compare several proportions. The Mann-Whitney U test was applied to determine whether two distributions differed in location. All P values reported refer to two-sided tests.

RESULTS

The characteristics of the patient populations in the ABVD and the MOPP/ABVD trials are shown in table 1. Ages of the patients were similar in both

TABLE 1.—Characteristics of heavily pretreated patients with Hodgkin's disease

Characteristic*	ABVD group	MOPP/ABVD group
Total patients	27	18
Age in yrs		
Median	33	29
Range	20–52	20–59
Sex		
Males	16	13
Females	11	5
Histology		
Nodular sclerosis	14	7
Mixed cellularity	3	7
Lymphocytic predominance	1	0
Unclassified	9	4
Interval from diagnosis to treatment, in mos		
Median	63	36.5
Range	4–264	5–136
Disease-free interval immediately prior to diagnosis, in mos		
Median	0	12
Range	0–2	0–104
Sites of involvement		
Nodal only	7 (26%)	8 (44%)
Extranodal + nodal	20 (74%)	10 (56%)
Bone	7	5
Liver	8	4
Lung	8	3

*Unless indicated otherwise, values = No. of patients.

TABLE 2.—Prior activity of Hodgkin's disease in patients treated with MOPP/ABVD and ABVD*

Prior activity	ABVD group	MOPP/ ABVD group
< 3 relapses	0 (0)	7 (39)
≥ 3 relapses	7 (26)	3 (17)
Continuous disease activity	20 (74)	8 (44)

*Values = No. of patients (%). $P < 0.002$.

TABLE 3.—Response in heavily pretreated patients with Hodgkin's disease

	ABVD group	MOPP/ABVD group
Total No. of patients	27	18
No. of patients with—		
CR (duration in mos)	1 (8)	9 (5–45+)
PR (duration in mos)	16 (1–31)	4 (5–25)
No change	1	0
Disease progression	9	5

trials. There was a slight preponderance of males over females and of the mixed cellularity histology in the MOPP/ABVD trial. A more striking difference was the median interval between initial diagnosis and start of salvage treatment, which was 63 months for the ABVD trial and 36.5 months for the MOPP/ABVD trial ($P < 0.05$). Twenty of 27 patients (74%) in the ABVD trial and ten of 18 patients (56%) in the MOPP/ABVD trial had extranodal disease when re-treatment was begun.This difference was not statistically significant. All of the patients in the ABVD trial had had either continuous active disease (74%) or more than three relapses (26%) prior to treatment, while 39% of the patients in the MOPP/ABVD trial had fewer than three relapses (table 2).

The responses to ABVD and MOPP/ABVD treatments are shown in table 3. The patients failing to achieve a CR with MOPP/ABVD were similar to those treated with ABVD alone in their poor response to prior treatment. Of the nine patients who achieved a CR with MOPP/ABVD, six had a history of fewer than three prior relapses, three had had three or more prior relapses, and none had a prior history of continuous active disease. In contrast, of the nine patients who failed to achieve a CR, eight had prior histories of continuous active disease, while only one had achieved a CR with previous chemotherapy. All nine of the patients achieving a CR with MOPP/ABVD had had a disease-free interval of at least 11 months immediately prior to re-treatment, while the disease-free interval had been either < 11 months or nonexistent in eight of the nine patients who failed to achieve a CR. Thus, the patients most likely to fail to achieve a CR with MOPP/ABVD were those with a history of either continuous active disease despite prior treatment or a CR of < 11 months' duration immediately prior to treatment.

Although it was not significant, a trend was observed in the MOPP/ABVD trial suggesting that patients with only nodal sites of recurrence have a higher CR rate than those with extranodal disease. Six of the nine patients achieving a CR had only nodal sites of relapse, as compared with only two of nine who failed to achieve a CR ($P = 0.153$).

The survival of patients from the time of retreatment in the MOPP/ABVD trial was superior to that of patients in the ABVD trial ($P = 0.004$, fig 1). However, after excluding patients with fewer than three relapses and while adjusting for prior response, the difference between the survival patterns of the two protocols was no longer significant ($P = 0.14$).

DISCUSSION

Re-treatment of heavily pretreated patients with Hodgkin's disease using combination chemotherapy has met with varying success, as our ABVD and MOPP/ABVD trials demonstrated. The CR rates reported for variants of ABVD alone among patients relapsing after or failing prior chemotherapy have ranged between 4% and 62%. Since the differences in these ABVD regimens were minor, it seemed likely that the patient groups treated by the various investigators were not comparable. This prompted us to look for differences in the populations of heavily pretreated patients with Hodgkin's disease who were treated in our ABVD and MOPP/ABVD trials. It seemed unlikely to us that the superior results of the MOPP/ABVD trial could be due solely to the addition of MOPP to ABVD, since all patients had been previously treated with variants of MOPP. We hoped that if differing prognostic factors emerged from the analysis of our two trials and those reported in the literature, we might be better able to define which heavily pretreated patients with Hodgkin's disease would be most likely to attain a second long-term CR with intensive combination chemotherapy.

The major difference between the patients in the two trials was the activity of their disease prior to treatment. The difference in median disease duration prior to salvage treatment was a reflection of this difference in disease activity. Twenty of the 27 patients in the ABVD trial never achieved a CR

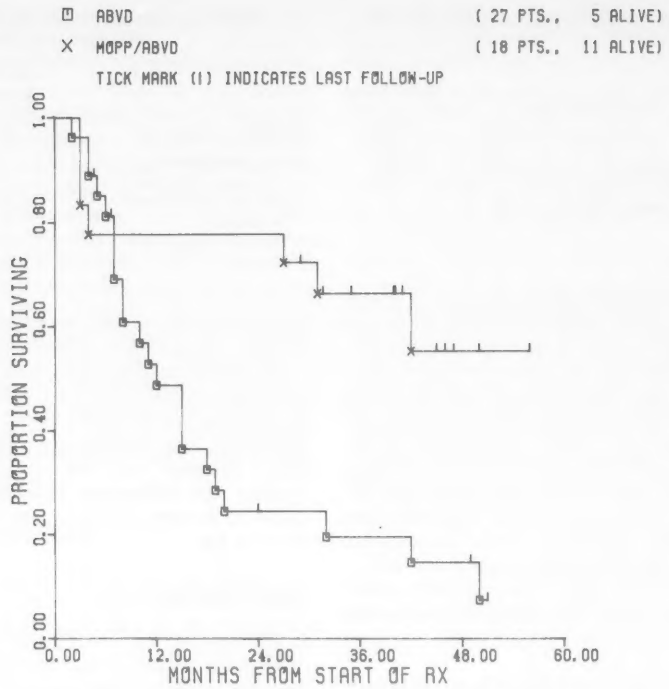

FIGURE 1.—Survival of patients from start of salvage treatment with ABVD and MOPP/ABVD.

prior to re-treatment, and the longest disease-free interval immediately prior to re-treatment in the remaining seven patients who had a previous CR was 2 months. The patients who failed to achieve a CR in the MOPP/ABVD trial were similar to those in the ABVD trial. Eight of the nine patients who failed to achieve a CR on MOPP/ABVD also had never achieved a CR on prior treatment, while all nine of the patients achieving a CR had a disease-free interval of at least 11 months after a prior CR immediately before treatment with MOPP/ABVD. Fisher and co-workers (10) reported that 59% of patients re-treated with MOPP after relapse from an initial CR attained a second CR with a median duration of 21 months. Fourteen of 15 patients whose initial CR lasted at least 12 months achieved a second CR with MOPP, whereas a second CR was attained in only five of 17 patients with a disease-free interval of < 12 months. It is thus possible that the CR rate of our patients treated with MOPP/ABVD might have been similar if they had been treated instead with MOPP alone.

Of the nine patients achieving a CR with MOPP/ABVD, six had only nodal sites of relapse, as compared with two of nine failures. This trend toward a greater likelihood of attaining a CR for patients with only nodal sites of relapse is also reflected in the results of other salvage regimens in the literature. In the ABVD trial of Santoro and Bonadonna (4), 62% of patients (13 of 21) had nodal disease only. Seventy-seven percent of patients (ten of 13) with nodal disease achieved CR as compared to a 38% CR rate (three of eight) for patients with extranodal disease. The sites of relapse prior to the start of bleomycin, dacarbazine, vincristine, prednisone, and doxorubicin (B-DOPA) are not provided by Lokich et al (11), who reported a 60% CR rate. In contrast, extranodal disease was present in 85% of the patients (23 of 27) treated with ABVD in the study by Krikorian et al (6), who reported a 22% CR rate, and in 93% of the patients (38 of 41) in the ABVD trial of Sutcliffe et al (7), who reported a CR rate of 7%. Extranodal disease was present in 74% of the patients in our trial with ABVD. The Stanford

University group (12) reported that the substitution of CCNU for dacarbazine in ABVD (B-CAVe) resulted in a 50% CR rate in heavily pretreated patients with Hodgkin's disease, which represented an improvement over the results with ABVD reported for their group by Krikorian et al (6). Although the precise number of patients with nodal disease only is not supplied in the B-CAVe report (12), it appears that there was a lower incidence of extranodal disease than in their ABVD trial. This difference may partially explain the differing results in the two trials. Lastly, in the report of Fisher et al (10) on re-treatment with MOPP, 68% of the patients (13 of 19) achieving a CR had nodal disease only as compared to 38% of patients (five of 13) who failed to respond or attained only a PR.

There are undoubtedly many factors which determine the success of the salvage treatment of relapsed patients with Hodgkin's disease. Most investigators have concentrated on the problem of drug resistance in patients previously treated with chemotherapy. The studies of Santoro and Bonadonna (4) and Lokich et al (11) demonstrated the usefulness of alternative drug combinations in treating MOPP failures and early relapses. The discrepancy in the results reported with ABVD and the contrast between our own ABVD and MOPP/ABVD results led us to examine patient characteristics, as was also suggested by Sutcliffe et al (7). The similarity of the CR rates and survival between patients with more than three prior relapses or prior continuous disease activity in both trials suggests that the overall differences in results may have been due to prior disease activity rather than to differences in the treatment regimens. It is hoped that future comparisons of salvage chemotherapy programs for Hodgkin's disease will be made between patient groups with comparable disease characteristics.

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Intermittent Continuous Iv Infusion of High-Dose Cyclophosphamide for Remission Induction in Acute Lymphocytic Leukemia[1]

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SUMMARY

Cyclophosphamide was administered to 42 patients with acute lymphocytic leukemia (ALL) as a daily continuous iv infusion at a dose of 400 mg/m²/day × 5 days; the courses of treatment were repeated every 3 weeks. Of the 42 patients entered, 21 achieved a complete response, two achieved a partial response, 12 failed to respond, and seven were considered to have early deaths. The response rate was 69.5% if only patients who received adequate trials are considered; mean duration of response was 18.5 weeks and mean survival time was 24.2 weeks. Twenty-three patients had relapsed after previous chemotherapy, and 19 patients were untreated for advanced high-risk cases of ALL; no difference was found in the response rates, durations of response, and survival times between these groups. No significant genitourinary toxicity occurred. Myelosuppression became the dose-limiting toxic effect. Continuous infusion of cyclophosphamide is a clinically effective method of ALL treatment and may have a role in the initial combination regimens for this hematologic malignancy.

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Cyclophosphamide is a class II cycle-specific agent with relatively less effect on resting stem cells than on proliferative lymphoma cells; stem cells are drawn from the resting phase into the proliferative state to repair marrow injury (1). Cyclophosphamide is unique among alkylating agents in that it is inactive in vitro, it requires activation to an alkylating metabolite by hepatic microsomes, and it has a platelet-sparing effect; the half-life of intact cyclophosphamide is 6.45 ± 1.1 hours (2). It is also unique among alkylating agents as an agent of proven effectiveness against acute lymphocytic leukemia (ALL), but unlike other agents of comparable activity in ALL and acute undifferentiated leukemia, cyclophosphamide has not been fully exploited in attempts at remission induction by combination chemotherapy (3).

In previously untreated patients, use of cyclophosphamide yields a 40% complete response (CR) rate, which is comparable to that of 6-mercaptopurine and inferior only to that of vincristine and to that of prednisone. In disease that is refractory to other agents, cyclophosphamide has been reported less effective, with an overall response rate of only 9% (3–6). However, it has been suggested that dose and schedule might influence the CR rate: the remission rate was 15% with a weekly oral dose of 10 mg/kg, 5% with a daily dose of 2 mg/kg, and 9% with sliding-scale dosages (7). Lately, use of cyclophosphamide has become common in the preparation of bone marrow transplant recipients, with doses of 45–60 mg/kg/day iv for 4 days found to be tolerable (8,9). In a study designed to evaluate the effectiveness of high-dose cyclophosphamide in inducing remission in children with acute leukemia (10), cyclophosphamide was given at a dose of 40 mg/kg/day for 4 days by rapid iv infusion (over 1

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hour) to 14 children with ALL: five of these patients achieved bone marrow remission that lasted 4–6 weeks.

These data provided us the rationale for the administration of maximally tolerated doses of cyclophosphamide in 120 hours of continuous iv infusion to patients with refractory ALL, using 6-hour dose fractionation, in an attempt to reach a maximal decrease in the leukemic cell population.

MATERIALS AND METHODS

All patients with histologically proven ALL who had failed prior remission induction therapy or who had relapsed after previous chemotherapy were eligible for this study. Later, a group of patients with far-advanced ALL or high-risk untreated ALL were included for the purpose of comparison. All patients had to have signed a consent form in accordance with our institutional policy.

Cyclophosphamide was administered as a daily continuous infusion at a dose of 400 mg/m²/day × 5 days. Each daily dose was fractionated into four 6-hour doses and was given in four 500-ml bottles of dextrose solution. Courses of therapy were repeated every 3 weeks depending on the recovery from myelosuppression. Patients received three courses of chemotherapy before they were considered to have failed. Responders were given repeated courses of chemotherapy until relapse; after a maximum of six courses, all treatment was interrupted.

All patients had a complete medical history and physical examination, cbc, urinalysis, creatinine, uric acid, and pretreatment bone marrow aspiration and biopsy. Cbc and platelet count were done daily during the study and twice weekly thereafter. A bone marrow examination was repeated before each course of chemotherapy.

A CR was recorded when a patient had 5% blast cells in the bone marrow, when the polymorphonuclear cell count was 1000/mm³, when the platelet count was 100,000/mm³, and when no clinical evidence of disease was found. A partial remission (PR) was recorded when the patient's bone marrow contained 6%–30% blast cells and the peripheral blood cell count became normal. Duration of remission was measured from the time the response occurred. Survival was measured from the start of therapy.

RESULTS

Forty-two patients with ALL were entered in this study. Twenty-three patients (group A) had received prior chemotherapy. All of these patients had been given a combination of vincristine and prednisone, and the 16 who achieved a CR were subsequently maintained with daily 6-mercaptopurine treatment and weekly methotrexate treatment. For reinduction after failure or relapse, four patients had also been given cytarabine combined with daunorubicin or 6-thioguanine plus vincristine and prednisone. The mean age for this group was 11.4 years (range, 4–26), the mean duration of disease was 19.5 months (range, 3–96), and the mean wbc count was 52,000/mm³. Nineteen patients had had no previous therapy; all of them had far-advanced "high-risk" disease (group B). The mean age for this group was 23.8 years (range, 4–55), and the mean wbc count was 140,000/mm³; all of these patients had adenopathy, enlarged liver and spleen, and infiltration of bone, skin, and/or mediastinal mass (tables 1 and 2).

The response of the patients who received continuous infusion of cyclophosphamide is shown in table 3. Seven patients who died of rapidly progressing disease within the first 2 weeks of therapy were considered to have early deaths. Of all 42 patients entered in the study, 21 achieved a CR, two achieved a PR, and 12 failed to respond. If only patients who received adequate trials were considered, 60% achieved a CR and 5.7% achieved a PR (overall response rate, 65.7%). Table 3 also shows the response according to prior chemotherapy: 60% in group A (12 patients) and 73% in group B (11 patients) achieved a response. No difference in response was demonstrated between the groups ($\chi^2 = 0.67$).

Fourteen patients received a total of three courses of chemotherapy, six patients had four courses, three patients had five courses, and 12 patients had six courses. An M1 bone marrow was achieved after the first course of chemotherapy (13 patients), after the second course (seven), and after the fifth course (one).

The response duration ranged from 4 to 32 weeks (median, 9.3) for patients in group A and from 4 to 69 weeks (median, 20) for patients in group B. Mean survival time has been 30 weeks for responders and 11.5 weeks for nonresponders.

The toxicity of cyclophosphamide in this group of patients was mainly to the bone marrow. Of the 35 patients entered in the study, 25 developed severe leukopenia (< 1500 cells/mm³) or thrombocytopenia (< 100,000 cells/mm³), or both. Mean wbc count nadir was 700/mm³ (range, 50–1500 in group A and 250–1500 in group B). The wbc count nadir was reached on Day 11 (range, 5–18) by patients in group A and on Day 7 (range, 3–15) by patients in group B. Mean duration of leukopenia was 19 days (range, 8–37) for patients in group A and 20 days (range,

TABLE 1.—Pretreatment characteristics and response in 20 previously treated ALL patients

Patient No.	Sex	Age (yrs)	Duration of disease (mos)	Wbc count (cells × 10^3/mm^3)	Lymph node enlargement	Liver-spleen enlargement	Involvement of other tissues	Bone marrow pretreatment (% blast cells/ cells)	Bone marrow posttreatment (response/ cells)	M1 achieved after cycle No.	No. of cycles given	Duration of remission (wks)	Survival time (wks)
1	M	10	31	5.7	+	+	—	78/80	M1/40	I	4	18	34
2	M	11	13	320.0	+	+	—	47/70	M3/40	–	5	—	15
3	M	9	12	14.0	+	+	Mediastinum	86/100	M3/90	–	3	—	7
4	F	17	11	192.0	–	–	—	92/100	M3/100	–	3	—	6
5	M	13	6	53.1	–	–	—	53/100	M2/30	–	4	4	26
6	M	10	60	18.0	–	–	—	97/90	M1/20	II	6	7	22
7	M	7	28	20.1	+	+	—	95/100	M3/60	–	3	—	10
8	M	18	4	60.0	–	–	—	84/100	M1/40	V	6	6	47
9	F	13	96	3.0	–	–	Kidneys	93/100	M1/40	II	6	8	19
10	M	15	13	13.2	–	–	Bones	50/100	M1/10	I	3	8	12
11	F	18	8	39.0	–	–	—	77/100	M1/40	II	6	32	43
12	F	15	1	31.8	+	+	Soft tissue	78/100	M1/70	I	3	8	15
13	F	8	30	8.7	–	–	Bones	81/100	M1/50	II	6	15	28
14	M	4	18	30.0	+	–	—	95/100	M3/100	–	3	—	12
15	F	8	4	4.5	–	+	—	92/100	M3/80	–	3	—	8
16	F	5	17	64.0	–	–	Bones	37/100	M1/30	I	6	45	47
17	F	13	6	4.8	+	+	—	90/90	M1/30	I	3	4	7
18	M	14	14	6.8	+	–	Testis	24/80	M1/60	I	4	30	39
19	F	10	10	83.8	–	+	CNS	82/90	M3/30	–	3	—	19
20	F	10	11	78.0		+	—	66/100	M3/30	–	3	—	8

TABLE 2.—Pretreatment characteristics and response in 15 previously untreated ALL patients

Patient No.	Sex	Age (yrs)	Duration of disease (mos)	Wbc count (cells × 10^3/mm^3)	Lymph node enlargement	Liver-spleen enlargement	Involvement of other tissues	Bone marrow pretreatment (% blast cells/ cells)	Bone marrow posttreatment (response/ cells)	M1 achieved after cycle No.	No. of cycles given	Duration of remission (wks)	Survival time (wks)
1	M	23	2	87.0	+	+	—	89/100	M1/30	II	6	69	83
2	F	22	1	279.0	–	+	Mediastinum and pleura	80/100	M3/80	–	4	—	12
3	F	33	3	106.0	–	+	Bones	90/100	M2/30	–	5	8	19
4	F	43	1	80	+	+	—	97/100	M3/100	–	3	—	27
5	M	43	5	335.0	+	+	Skin	60/100	M3/100	–	3	—	15
6	F	55	3	110.0	–	–	—	95/100	M1/15	II	6	15	25
7	M	47	1	88.0	+	–	—	94/100	M1/40	I	6	20	35
8	M	15	2	220.0	+	+	Mediastinum and pleura	63/100	M1/40	I	6	18	20
9	M	12	1	52.4	+	–	Skin	91/100	M1/30	I	6	26	30
10	F	29	3	32.0	+	–	Breasts	96/100	M1/30	I	6	16	19
11	F	4	1	2.0	+	+	Mediastinum	50/100	M1/40	II	6	20	35
12	M	9	1	424.0	+	+	Mediastinum and pleura	85/100	M1/20	I	4	4	18
13	F	36	2	100.0	–	–	—	90/100	M3/100	–	3	—	6
14	M	4	1	136.0	+	–	Mediastinum	88/100	M1/20	I	5	13	32
15	F	22	1	53.0	+	+	—	90/100	M1/25	I	6	33	36

TABLE 3.—Response according to prior therapy

Prior therapy	No. of patients entered	No. of patients with— Early death	CR	PR	No response	Median duration of CR + PR in wks (range)	Median survival time in wks (range)
Group A (yes)	23	3	11	1	8	9.3 (4–32)	17 (5–47)
Group B (no)	19	4	10	1	4	20.0 (4–69)	26.6 (18–83)
Totals	42	7	21	2	12	15.0 (4–69)	20.0 (5–83)

TABLE 4.—Myelosuppression with continuous infusion of cyclophosphamide

Group	Wbc count* Nadir (cells × 10^3/mm^3)	Nadir Day	Duration of leukopenia (days)	Platelet count* Nadir (cells × 10^3/mm^3)	Nadir Day	Duration of thrombocytopenia (days)
A	0.7 (0.05–1.5)	11 (5–18)	19 (8–37)	30.0 (18.0–60.0)	10 (5–17)	16 (10–38)
B	0.7 (0.25–1.5)	7 (3–15)	20 (7–100)	43.0 (20.0–80.0)	12 (3–20)	14 (8–37)

*Values = mean (range).

7–100) for patients in group B. The mean platelet count nadir was 30,000/mm^3 (range, 18,000–60,000) for group A and 43,000/mm^3 (range, 20,000–80,000) for group B. Platelet count recovery occurred after 16 and 14 days, respectively (table 4).

Anorexia, nausea, and/or vomiting were observed in 13 patients (37.1%), and alopecia was observed in 27 (77.1%). Twenty patients developed fever that was attributed to infection and received antibiotics during or subsequent to the period of cyclophosphamide administration. Macroscopic hematuria was not seen in any patient, but microscopic hematuria was detected in four patients during a routine urinalysis.

DISCUSSION

This study indicates that continuous infusion of cyclophosphamide for 5 days is a clinically effective chemotherapy in patients with ALL. Cyclophosphamide given orally in a single daily dose or iv weekly has been reported to induce remission in 40% of untreated patients and in 9% of patients resistant to previous chemotherapy (4–6). It has also been observed that 24% of patients receiving daily regimens attained an M1 bone marrow; in that experiment, a daily regimen was felt to be more effective than a weekly one (3). Another study was 15% with the weekly dose and 5% with the daily dose (7). More recently, cyclophosphamide was given at a dose as high as 40 mg/kg/day for 4 days by rapid iv infusion, and bone marrow remission was attained in five of 14 children with ALL (10). When cyclophosphamide is given as in this study, ie, at a dose that is 50% lower but in a continuous 120-hour infusion, the CR rate is 50% of all patients entered, a figure which may suggest superiority to other results so far reported. This favorable result could be attributed to the technique of administration.

No significant difference was found in the response rate between group A (previously treated patients) and group B (untreated patients), nor was a difference found in the duration of the disease-free interval or in the survival time between the two groups. These results are clearly different from those reported by other investigators (4–6).

Courses of chemotherapy were well-tolerated. Myelosuppression, the dose-limiting toxic effect, was not more pronounced in previously treated patients than in untreated patients. Hemorrhagic cystitis was not present in any patient, which may be attributed to the hydration administered.

We conclude that our favorable results could be due to the method of administration of the drug, although it is also possible that other factors are involved. Thus, the value of continuous infusion of cyclophosphamide can be established only by a randomized trial comparing that method of administration to an iv bolus regimen with equivalent dosages. The clinical pharmacology of cyclophosphamide indicates that the plasma level of the drug and the subsequent exposure of rapidly proliferating cells to it can be prolonged when cyclophosphamide is

administered in fractionated doses by continuous infusion. The results of our studies indicate that continuous infusion of cyclophosphamide may have a role in the initial combination regimens for some hematologic malignancies.

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Simultaneous Treatment With Cisplatin and Radiation Therapy for Advanced Solid Tumors: A Pilot Study[1]

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SUMMARY

To study the toxicity and efficacy of simultaneous cisplatin chemotherapy and radiation therapy, 13 patients with metastatic solid tumors were treated with cisplatin on a weekly, outpatient basis during palliative radiation therapy. The dose of cisplatin ranged from 20 to 50 mg/m² weekly, with most patients receiving 40 mg/m². Radiation therapy was administered in a variety of dose-fraction schedules. Toxic effects were moderate and consisted of emesis (12 patients), transient elevation of BUN (three patients), myelosuppression (three patients), and radiation reactions (two patients). Twelve of 13 irradiated lesions (91%) responded with at least a 50% reduction in biperpendicular diameter. Four of the six patients with metastatic melanoma had complete regression of treated lesions; another melanoma patient had a partial response. Responses were also seen in patients with mesothelioma, bladder cancer, squamous cell carcinoma of the head and neck, and oat cell lung cancer. Only one responding patient has had disease progression in the treated field after 2+ to 7+ months of followup; two other patients have died of disseminated disease. Weekly cisplatin administration during radiotherapy deserves further evaluation, especially in the primary management of unresectable tumors that are responsive to cisplatin alone.

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Cisplatin has been under clinical investigation as an antitumor drug since 1971. The drug has an established role in the management of disseminated testicular and ovarian cancers and is also active in squamous cell carcinomas of the head and neck, lung, and cervix, in malignant melanoma, in bladder cancer, and in oat cell lung cancer (1).

In addition to tumor cytotoxicity, apparent radiosensitization properties of cisplatin have been observed in vitro and in vivo (2,3). Although the mechanism(s) of cisplatin-radiation interaction(s) is poorly understood,[3] the minimal overlap in toxic effects makes the combination of these modalities an attractive therapeutic approach to a variety of unresectable solid tumors. In May 1979, we started a pilot study to evaluate the toxicity and efficacy of weekly cisplatin given during radiation therapy to a variety of patients with metastatic solid tumors. The weekly schedule was empirically selected in an attempt to maintain tissue levels of cisplatin for the duration of radiation therapy.

MATERIALS AND METHODS

Patient eligibility for this study included the following: histologically proven unresectable or metastatic solid tumor that was measurable and not

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TABLE 1.—Characteristics of 13 patients receiving simultaneous cisplatin and radiation therapy

Patient No.	Disease	Area treated	Radiation therapy schedule*	Source	TDF†	Cisplatin schedule (mg/m² × wks)	Response of treated lesion	Status
1	Melanoma	Chest wall	3000/10/14; 2-wk rest; 2000/10/14	Cobalt	88.8	40 × 4	CR, 7 mos	Died with disseminated disease, 7 mos
2	Metastatic carcinoma	Abdominal mass	2000/5/7	Cobalt	48	50 × 2	Inevaluable	Died after 2 wks of disseminated disease
3	Melanoma	Soft tissue mass	4500/9/24	Linac, 10 MeV	112	50 × 4	CR, 6+ mos	No evidence of disease, 6+ mos
4	Melanoma	Brain	3300/11/14 + 800/4/6	Linac, 10 MeV	68	40 × 4	CR, 2 mos	Died with disseminated disease, 2 mos
5	Melanoma	Soft tissue mass	5000/17/33	Cobalt	100	40 × 4	PR, 8+ mos	Alive with disease, 8+ mos
6	Oat cell lung cancer	Lung	4000/20/33	Cobalt	66	40 × 3	CR, 5 mos	Died with disseminated disease, 5 mos
7	Squamous cell cancer of head and neck	Oropharynx	6400/32/49	Neutrons and Linac, 10 MeV	105	50 × 5	CR, 5+ mos	Alive with other metastases, 5+ mos
8	Melanoma	Skin	3300/7/30	Electrons, 7 MeV	75	40 × 3	Minor response, 1 mo	Alive with disease, 3+ mos
9	Melanoma	Pelvic mass	3600/20/39	Linac, 10 MeV	56	40 × 4	PR, 3+ mos	Alive with other metastases, 3+ mos
10	Melanoma	Foot	3000/10/13; 2-wk rest; 1200/4/13; 2-wk rest; 800/4/14	Cobalt	92	20 × 4	CR, 7 mos	Died with disseminated disease, 8 mos
11	Pleural mesothelioma	Pleura	2000/13/19 (18 × 22-cm field)	Linac, 10 MeV	28	40 × 3	PR, 4+ mos	Pleural scarring on chest roentgenogram; no symptoms, 4+ mos
12	Squamous cell cancer of nasopharynx	Neck	1400/7/9 (after prior radiotherapy)	Cobalt	24	40 × 3	CR, 3+ mos	No evidence of disease, 3+ mos
13	Bladder cancer	Abdominal mass	3200/10/14	Linac, 10 MeV	70	50 × 2	PR, 3+ mos	Alive with other metastases, 3+ mos

*Values = No. of rads/No. of fractions/No. of days.
†TDF = time-dose-fraction factors.

deemed curable by standard therapies, no prior cisplatin chemotherapy, a life expectancy of at least 6 weeks, a creatinine clearance of ≥ 40 ml/minute, and informed consent.

Cisplatin was given weekly during radiation therapy at doses ranging from 20 to 50 mg/m², with most patients receiving 40 mg/m². Patients were usually hydrated on Monday morning in the outpatient clinic with 1000 ml of iv fluids, followed by 12.5 g of mannitol by iv push and then infusion of the cisplatin over 30 minutes. Subsequent doses of cisplatin were adjusted for increases in BUN (to > 20 mg/100 ml) or creatinine (> 1.4 mg/100 ml) or for myelosuppression.

Radiation therapy was given in a variety of dose fractionation schedules commonly used in the palliation of solid tumors (table 1). One patient with widespread cutaneous metastases received electron beam radiation; another patient with squamous cell carcinoma of the oropharynx was treated with neutrons and photons ("mixed beam" therapy).

Complete response (CR) was defined as disappearance of the irradiated lesion for at least 1 month. Partial response (PR) was defined as a > 50% reduction in the product of two bidirectional measurements, persisting for at least 1 month. Although metastases outside of the radiation therapy port were also measured, only irradiated lesions were used to determine response.

RESULTS

The pretreatment characteristics are summarized in table 1. Thirteen patients were entered in the study, including seven with melanoma, two with squamous cell carcinomas of the head and neck, and one each with mesothelioma, bladder cancer, recurrent oat cell lung cancer, and metastatic carcinoma from an unknown primary site.

No patient had prior cisplatin chemotherapy; one patient (No. 12) had suboptimal prior radiation therapy to the area of recurrent disease.

CRs were observed in seven patients (four of seven patients with melanoma, two of two with squamous cell carcinomas, and the one with refractory oat cell lung cancer). A patient with pleural mesothelioma had a dramatic relief of dyspnea and pleuritic pain but still had residual pleural changes on chest roentgenogram. Four patients had PRs, one had < 50% reduction in tumor, and one was inevaluable for response (early death). The overall response rate was 91%. The only melanoma patient who did not respond had multiple cutaneous metastases on the upper arm and received cisplatin during electron-beam radiation therapy. Responses in melanoma were seen in visceral as well as soft tissue lesions (table 1).

TABLE 2.—Toxicity of simultaneous cisplatin and radiation therapy

Toxic effect	No. of patients affected
Vomiting after cisplatin	12
Radiation toxicity	
Cutaneous	2
Mucositis	1
Esophagitis	1
Hematologic toxicity	
Anemia (< 10 g/100 ml of Hgb)	1
Neutropenia* without fever	3
Renal toxicity (BUN > 20 mg/100 ml)	3

*Granulocytes < 1000/mm³.

Only one patient (No. 10) who achieved a CR had a recurrence within the irradiated field. Several patients, however, have developed progressive disease in unirradiated areas. Three patients had measurable metastases outside the radiation port. Patient 7 had a stabilization of pulmonary metastases for 3 months. Patient 9 had skin metastases that progressed during therapy. Patient 10 had a subcutaneous nodule that had a < 50% reduction for 2 months.

The toxicity of this regimen was moderate (table 2). Vomiting after cisplatin was almost universal but generally persisted for < 6 hours. Reversible radiation reactions were noted in four patients; in each instance the treating physician felt that the cisplatin had not increased the severity or duration of toxicity; ie, the observed toxicity was not greater than might be expected with radiation therapy alone. Renal toxicity (BUN > 20 mg/100 ml) was mild and readily reversible.

DISCUSSION

Several investigators have studied the radiosensitizing effects of cisplatin in tissue culture. Douple and Richmond (2) demonstrated that V-70 Chinese hamster cells were sensitized to radiation therapy by cisplatin. The effect was most dramatic in hypoxic cells. Similar effects were also noted in transplantable mouse breast adenocarcinoma and in rat brain tumors (3). The mechanism of this sensitization is not clear, but at least one investigator suggests that it may be due to inhibition of DNA repair mechanisms by cisplatin (*see* footnote 3).

Little is known about relative tissue concentrations of cisplatin in man. It is generally believed

that there is only minimal uptake of the drug in normal tissues. Tumors that are responsive to cisplatin have the ability to transport the drug intracellularly. While the initial half-life of cisplatin is measured in hours, its second half-life (ie, the measure of tissue distribution) may be several days (4). For this reason we selected a weekly schedule for cisplatin administration.

Theoretically, cisplatin has many advantages over other radiosensitizers. First, the drug is a potent cytotoxic agent per se. Thus, increased cell kill in the primary tumor might occur simultaneously with control of micrometastatic disease. Second, if cisplatin is not concentrated in normal tissues, then it is reasonable to expect that there will be less radiation toxicity in surrounding tissues compared to the treated tumor. Third, the common toxic effects of cisplatin are well-described and generally do not overlap with toxic effects of radiation therapy.

This study demonstrated that the combination of weekly cisplatin and ionizing radiation is a clinically feasible regimen. While the responses observed may have been due to either modality alone, the responses noted in six of seven patients with refractory invasive melanoma suggests the possibility of therapeutic synergism. Prospective randomized trials comparing well-defined radiation therapy alone or with concurrent weekly cisplatin will be required to definitively answer this question. Such trials may be reasonable in several tumors, including invasive melanoma; advanced lung, bladder, cervical, esophageal, and head and neck cancers; and mesothelioma.

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Pulmonary Disease as a Complication of Chlorozotocin Chemotherapy[1]

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SUMMARY

Two of 200 patients treated with chlorozotocin have evidenced interstitial pulmonary disease. The cumulative doses of chlorozotocin were 480 and 670 mg/m² when symptoms developed. Pulmonary function tests in both patients showed impaired diffusion, and an alveolar-arterial gradient which worsened with exercise. Open-lung biopsy in one patient showed typical interstitial inflammation and early fibrosis, and excluded lymphangitic spread of tumor. Discontinuation of chlorozotocin resulted in either improvement or stabilization of the pulmonary disease.

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Several classes of antineoplastic drugs, and, in particular, the alkylating agents, have been recognized as potential pulmonary toxins (1–3). Two types of pulmonary toxicity have been described: direct cytotoxic injury, usually due to bleomycin or alkylating agents, and allergic interstitial pneumonia or hypersensitivity pneumonitis, reported with methotrexate, and, rarely, with bleomycin or procarbazine.

Of the alkylating agents in active clinical use, busulfan and cyclophosphamide have been implicated most frequently in the production of pulmonary fibrosis. However, recently the nitrosoureas BCNU and methyl-CCNU have also been reported to cause lung toxicity. Whereas interstitial infiltrates during BCNU therapy were reported in the literature as early as 1966 (4), a cause and effect relationship was not clearly demonstrated until 1976 (5). At the present time, 13 cases have been documented in which administration of BCNU appeared to result in pulmonary fibrosis (3). The cumulative doses were 580–2100 mg/m². Most of these patients died of the drug-related pulmonary complications. Autopsy findings consistently demonstrated nonspecific interstitial inflammation and fibrosis, indistinguishable from that produced by busulfan. Methyl-CCNU, given at a cumulative dose of 4100 mg/m², has thus far been implicated in only one case of pulmonary toxicity (6).

Because of the considerable structural similarities among the nitrosoureas, pulmonary toxicity might be anticipated from other members of their group. We report here two patients in whom administration of the nitrosourea chlorozotocin is correlated with the development of interstitial pulmonary disease.

CASE REPORTS

Patient 1

Patient 1 is a 68-year-old woman who presented in March 1979 with a perforated cecal carcinoma and biopsy-proven metastatic disease in the liver. Chest roentgenogram disclosed multiple pulmonary metastases. She had an uneventful postoperative course and was asymptomatic when discharged. In April 1979 she started receiving combination chemotherapy with 5-FU (325 mg/m² iv daily for 5

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days) and chlorozotocin (100 mg/m² iv, repeated at 28-day intervals). After two cycles a reduction in the size and density of the pulmonary nodules was seen on chest roentgenogram and the liver scan demonstrated a partial response. Therapy was continued and she remained asymptomatic until November 1979 when, after seven cycles of chemotherapy, she reported the onset of dyspnea during exertion. This had developed over about a 3-week period and limited her to climbing about one flight of stairs, whereas she had previously had no restriction. On examination, a few diffuse, dry, inspiratory rales were noted. There were no findings suggesting cardiac failure, nor was there any history of cardiac disease. Chest roentgenogram showed continued regression of the metastatic nodules, but with a diffuse increase in interstitial pulmonary markings. There was no exposure to known pulmonary toxins. Pulmonary function tests were performed, which demonstrated normal static volumes except for mildly reduced residual volume (72% of predicted), reduced flows, and a reduction in carbon monoxide diffusing capacity (DL_{CO}) to 59% of normal (table 1). During exercise, her alveolar-arterial oxygen gradient increased from 24 to 52 mm Hg, and her arterial pO_2 decreased by 31 mm Hg, from 88 to 57. An open biopsy of the lingula was performed and showed interstitial inflammation and an early fibrosis, consistent with drug-induced pulmonary toxicity. No tumor was found in the specimen. Chlorozotocin was discontinued but 5-FU chemotherapy was continued at monthly intervals. The patient's dyspnea during exertion had essentially resolved 2 months later, although disease progression was again noted in her metastatic pulmonary nodules.

Patient 2

Patient 2 is a 40-year-old woman who in 1977 noted an enlargement of a nonpigmented nevus which had been present for several years on her left upper arm. Biopsy revealed a highly anaplastic amelanotic melanoma but the patient refused the recommended wide excision. In September 1978 local disease recurrence was noted in association with a persistent nonproductive cough. Chest roentgenogram (fig 1a) showed numerous metastases, roughly 1–3 cm, in both lungs. After excisional biopsy of the arm lesion, the patient started receiving single-agent chemotherapy with chlorozotocin at a dose of 120 mg/m² given iv at 4–6-week intervals. The therapy was initially well-tolerated, with only transient nausea and vomiting and nominal myelosuppression. A prompt regression of the lung nodules was observed. However, in January 1979 she reported increasing fatigue with chest pressure and dyspnea during exertion. At that time there had been a complete resolution of the metastatic nodules, but an increase in interstitial markings was visible on chest roentgenogram (fig 1b). Liver and bone scans were negative. Pulmonary function tests were performed and showed essentially normal static volumes, flows, and normal resting blood gasses; however, the DL_{CO} was significantly decreased to 56% of the predicted value (table 1). With exercise the alveolar-arterial O_2 gradient increased from 1 to 15 mm Hg and the arterial pO_2 decreased by 8 mm Hg. Although consistent with a pathologic interstitial process, the abnormality was relatively mild and was not felt to justify discontinuation of the otherwise successful chemotherapy. While being closely monitored, she received a fifth and sixth dose of chlorozotocin and pulmonary function tests were repeated in May 1979. These showed further deterioration, with the DL_{CO} now 43% of the predicted value. The dose of chlorozotocin was decreased to 90 mg/m² and two additional doses were given; therapy was then discontinued. DL_{CO} stabilized at approximately 50% of the predicted value and residual volume stabilized at approximately 75% of the predicted value; there has been no further symptomatic progression of her pulmonary disease. Her chest roentgenogram has remained free of metastatic nodules 6 months after discontinuation of therapy (15 months after initiation of therapy), but she has had a recurrence in the brain.

PULMONARY FUNCTION TESTS

The pulmonary function tests performed on the two patients are shown in table 1. The significant findings are a mildly reduced residual volume, an impaired diffusion, and an alveolar-arterial gradient of oxygen which significantly widens with exercise. These are the characteristic findings of interstitial pneumonitis or fibrosis. In patient 2, the test was performed after administration of cumulative doses of 480 and 600 mg/m² of chlorozotocin, and demonstrated progression of the DL_{CO} impairment and residual volume reduction. The patient would not permit blood gas measurements during the second test.

PATHOLOGY (PATIENT 1)

Aside from a uniformly homogeneous deep red appearance, no specific gross pathologic features were noted. Microscopic examination revealed a variable pattern. Many alveolar capillary walls were thickened, some with early collagen deposition, and were filled with mononuclear cells (figs 2 and 3).

TABLE 1.—Pulmonary function tests*

	Cumulative chlorozotocin (dose)		
	Patient 1 (670 mg/m²)	Patient 2 (480 mg/m²)	Patient 2 (600 mg/m²)
Forced vital capacity	107%	112%	108%
Residual volume	72%	84%	75%
TLC	92%	102%	96%
FEV_1/FVC	65%	83%	83%
DL_{CO}	59%	56%	43%
Resting arterial blood gas (ABG)	88/31/7.46†	113/30/7.50	Not performed
Exercise ABG	57/30/7.43	105/25/7.40	
100% O_2 ABG	524/23/7.55	587/22/7.44	

*Principal abnormalities are mildly reduced residual volume, significantly reduced DL_{CO} which in patient 2 deteriorated further with continued administration of chlorozotocin, and an alveolar-arterial gradient which increased significantly with exercise.

†Values = $pO_2/pCO_2/pH$.

While no granulomata were seen, in some areas covalescence of mononuclear cells mimicked a granulomatous inflammation. In addition to the interstitial pattern, an intra-alveolar cellular infiltrate composed of mononuclear cells was noted. Alveolar macrophages were prominently represented. Occasional areas of organizing exudate were present. No organisms (bacterial, fungal, or protozoal) were seen, nor were there any viral inclusions. There was no malignancy seen in the tissue examined.

DISCUSSION

Chlorozotocin is a synthetic sugar-linked nitrosourea closely related to the naturally occurring antineoplastic antibiotic streptozocin (7). It was designed to retain the reduced myelosuppressive characteristics of streptozocin, while maintaining the spectrum of activity observed with the chloroethyl class of antitumor agents (8). Chlorozotocin has demonstrated useful activity in a number of tumors in our series, including melanoma, gastrointestinal malignancies, lymphoma, and others, without the necessity of serious myelosuppression (9). This is well-demonstrated in patient 2, who achieved a complete regression of pulmonary metastases while maintaining essentially normal wbc and platelet counts during 9 months of continuous therapy.

Approximately 260 patients have now been treated with chlorozotocin at the Vincent T. Lombardi Cancer Center. Of these, approximately 60 patients were simultaneously treated wtih mitomycin C (in the 5-FU, doxorubicin, mitomycin C, and chlorozotocin protocol), an agent known to have the potential for pulmonary toxicity. The remaining 200 patients were treated with either chlorozotocin alone or combined with 5-FU. This patient group ranged in age from 21 to 81 years, with a median age of 56 years. The total cumulative dose ranged from 80 to 1300 mg/m². The underlying malignancies were primarily colon, melanoma, breast, and lung, although several other tumors were also represented.

We have now observed two patients in whom the administration of chlorozotocin has been associated with the development of interstitial pulmonary disease. One patient never received any other chemotherapeutic agent; in the other patient, only 5-FU was simultaneously administered and there was no previous chemotherapy. In spite of its widespread use, 5-FU has never been reported to cause pulmonary toxicity, although potentiation of another pulmonary toxin cannot be ruled out. Neither patient had received radiotherapy.

One patient was being treated for metastatic colon carcinoma and the other for metastatic melanoma; both were responding to therapy when pulmonary toxicity became evident. Symptoms of increasing dyspnea during exertion developed after cumulative administration of 480–670 mg/m² of chlorozotocin, and pulmonary function tests showed im-

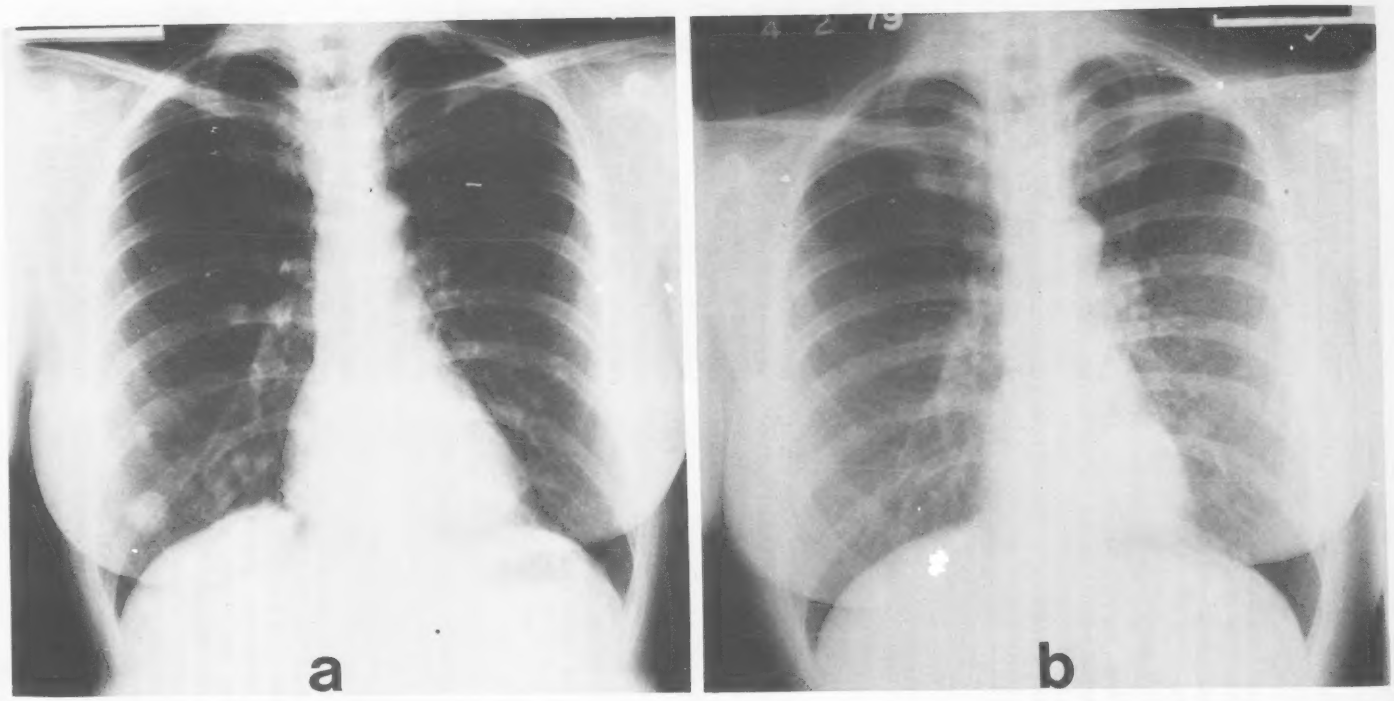

FIGURE 1.—Patient 2: chest roentgenogram before chlorozotocin therapy (a) and after a cumulative chlorozotocin dose of 600 mg/m^2 (b).

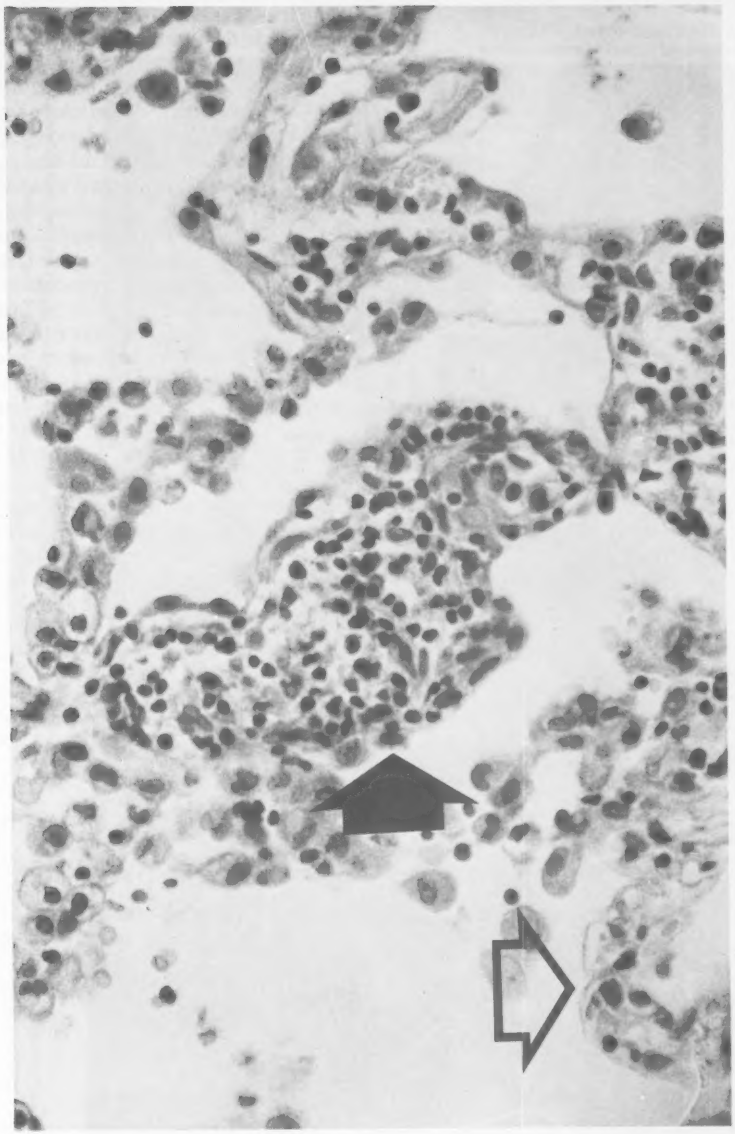

FIGURE 2.—Patient 1: open lung biopsy. Substantial mononuclear infiltrate has widened the alveolar walls (solid arrow). Other alveolar walls are less impressively infiltrated (open arrow). Original magnification, × 125.

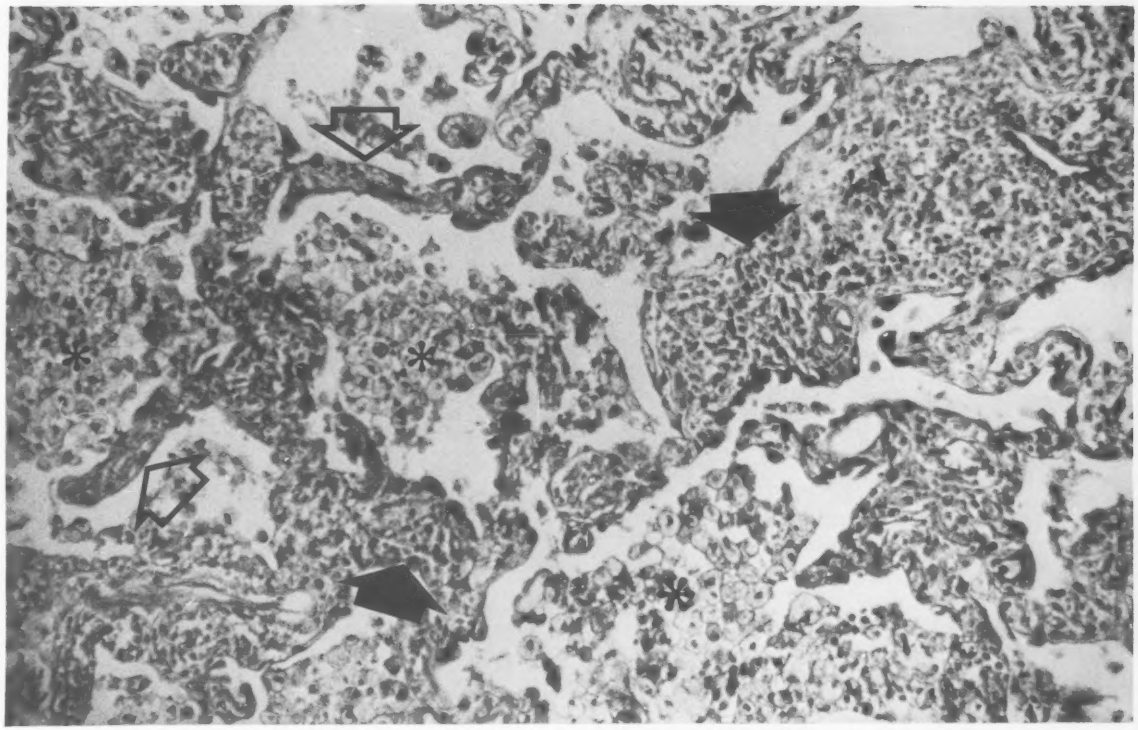

FIGURE 3.—Patient 1: open lung biopsy. Variable degree of alveolar wall infiltration (solid arrows) is intermingled with an intra-alveolar cellular reaction (asterisks). Early fibrous thickening of some alveolar walls is seen (open arrows). Original magnification, × 32.

paired diffusion, abnormal response to exercise, and mild reduction in residual volume. Open lung biopsy in one patient showed interstitial inflammation, early fibrosis, and other changes typical of drug-induced interstitial pulmonary disease. When chlorozotocin administration was discontinued, both patients experienced a remission of their pulmonary symptoms, and further progression of their interstitial processes was not detected. This would suggest that neither patient had yet reached the stage of advanced fibrosis.

The pulmonary histopathologic findings and occurrence of toxicity after several doses of the drug are suggestive of a direct cytotoxic effect on the lungs similar to that produced by other alkylating agents, and more commonly by bleomycin. The DL_{CO} was markedly reduced in both patients and appears to be more sensitive than spirometry in detecting this form of toxicity, as pointed out by Comis et al (10).

We believe that these cases represent pulmonary toxicity induced by the chemotherapy, and that chlorozotocin may be added to the growing list of chemotherapeutic agents having the potential for induction of interstitial pulmonary disease.

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Doxorubicin and Dactinomycin Therapy for Advanced Sarcomas[1]

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SUMMARY

Doxorubicin and dactinomycin were administered to 25 patients with advanced sarcomas. Twenty-one were evaluable for response. There were six responses (two complete and four partial) (28%) and eight improvements. Responders survived longer than nonresponders, while those with improvements had intermediate survival. Toxic effects included leukopenia in 35% of administered courses, mucositis in 50% of the patients, and congestive heart failure in 21% of the patients. Plasma doxorubicin kinetics did not correlate with excess toxic effects in four patients studied. The response rates and durations of survival obtained with this combination are comparable to those obtained with doxorubicin alone. The marked increase in toxicity makes this combination less desirable than treatment with doxorubicin alone.

[Cancer Treat Rep 65:231–236, 1981]

Advanced soft tissue sarcomas in the adult population continue to be difficult neoplasms to treat for prolonged periods. Surgical resection continues to be the most effective primary therapy. Unfortunately, primary therapy frequently fails to adequately control local disease, and, in many instances, widespread disease becomes evident within 1 year of primary diagnosis.

Patients with disseminated disease face a poor prognosis. Currently available chemotherapy offers short palliation (1) at best and provides impetus to search for more effective combinations of drugs or new agents. For adult sarcomas, doxorubicin remains the mainstay of treatment (1–4). Other active single agents have included DTIC (1,5), cyclophosphamide, and vincristine (6–8). For pediatric sarcomas, major drug activity for embryonal rhabdomyosarcomas with the single agents dactinomycin, vincristine, and cyclophosphamide (6,9,10) has been found. Combination chemotherapy with these drugs has produced improvement over single agents (5,11). Current drug combinations with doxorubicin offer minimal advantage over doxorubicin alone and provide short-term palliation at best.

Two single active drugs, doxorubicin and dactinomycin, may provide improved palliation when given together. Although animal studies (T. Corbet, personal communication) have not shown significant synergism with these drugs, synergism is theoretically possible, as methods of DNA intercalation are different with these drugs (12). In this paper, we report the results of a pilot study of doxorubicin and dactinomycin in a heterogeneous group of patients with advanced sarcomas.

MATERIALS AND METHODS

All patients referred to the Baltimore Cancer Research Program between February 1976 and February 1978 with advanced histologically documented soft tissue or bone sarcomas were entered into this protocol. Pretreatment evaluation consisted of a complete history and physical examination, cbc, platelet count, electrolytes, SMA-12, electrocardiogram, chest roentgenogram, BSP dye retention, and urinalysis. Additional roentgenograms, scans, and procedures were performed as necessary. Tumor measurements were assessed by measurement of mass size of any palpable lesions

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and by the cross-sectional perpendicular diameters of any visible lesions on scan or roentgenogram.

All patients were treated with doxorubicin (60 mg/m²) by iv infusion over 5 minutes through a running iv line on Day 1. Dactinomycin (300 μg/m²/day) was administered for 5 days on Days 8–12 through a running iv line. Courses were repeated every 28 days with cycles lengthened for myelosuppression, mucositis, or other toxic effects.

Doxorubicin was modified according to hepatic dysfunction. Dose modifications were determined by BSP dye retention (50% dose for 9%–15% BSP retention, 33⅓% dose for > 15% BSP retention) or pretreatment bilirubin (50% dose for bilirubin of 1.5–3.0 mg/100 ml, 33⅓% dose for bilirubin > 3 mg/100 ml [13]). Dactinomycin doses were reduced by one half if wbc count nadirs were < 1500/μl or platelet count nadirs were < 75,000/μl and if the nadirs lasted > 3 days. If the patient became febrile or required platelet transfusion, dactinomycin was reduced to one third the previous dose. Dactinomycin was discontinued if the wbc count was < 3000/μl or the platelet count was < 100,000/μl prior to any daily dose.

Complete remission was defined as the disappearance of all signs and symptoms of disease and the disappearance of all visible or measurable lesions for at least 4 weeks. Partial remission was defined as a > 50% reduction in the sum of the products or perpendicular diameters of all measurable lesions for at least 4 weeks accompanied by symptomatic improvement. Duration of response was defined as the number of weeks from the attainment of the objective response to the time of progression of measurable lesions and was documented by physical examination or laboratory evaluation or by the time of appearance of new lesions. Histologic evidence of disease progression or new lesions was obtained whenever possible. Improvement was defined as an objective response not fulfilling the criteria for partial remission and included definite stabilization of previously progressing disease for at least 4 weeks.

All patients received at least two courses of therapy before being considered unresponsive. Patients with any response or no progression (stable disease) continued to receive the combination until there was evidence of tumor progression, unacceptable drug toxicity, or cumulative dose of 540 mg/m² of doxorubicin.

Doxorubicin pharmacokinetic studies were performed in four patients receiving this combination. Twelve milliliters of heparinized blood was collected prior to and 30 minutes and 1, 2, 4, 8, 12, 24, 48, 72, and 96 hours following the administration of doxorubicin. Plasma collected was immediately stored at –20°C until the drug assays were performed.

Doxorubicin was assayed by fluorescence techniques previously described (14). The specimen was thawed slowly, then sonicated in ice for 15 seconds. Two milliliters of 75% acid-alcohol was added to 1 ml of plasma and the mixture was corked and vortexed for 15 seconds. After 18–24 hours in a dark refrigerator, the specimens were centrifuged at 15,000 rpm for 15 minutes at 4°C. The clear supernatant solution was removed and read against a standard curve on an Aminco (SPF-25) fluorometer at an excitation wavelength of 470 nm and a detection wavelength of 585 nm. Concentration times time ($C \times T$) was calculated using the trapezoidal rule.

RESULTS

Twenty-five patients were entered in this protocol. Twenty-four patients were evaluable for toxicity, while 21 were evaluable for response. Four patients were unevaluable for response: two had an incorrect diagnosis, one died prior to the administration of dactinomycin, and one had no measurable disease. Characteristics of the patient population studied are listed in table 1.

Fourteen of 21 patients obtained an objective response: two complete responses (CR), four partial responses (PR), and eight improvements. Although the overall improvement rate was 67%, only six of 21 patients obtained CR or PR, for a response rate of 28%. Median time to response was 6 weeks (average, 3–12). The response lasted from 12.5 to 62.5 weeks with a median duration of 28 weeks. Table 2 shows the response according to tumor type. Patients who responded received a median of nine cycles of chemotherapy. Among all treated patients, a median of six cycles of this combination were administered. The two patients with CR had response

TABLE 1.—Treatment characteristics of study group

Characteristic	
No. of patients treated	25
No. of patients evaluable for toxicity	24
No. of patients evaluable for response	21
Age in yrs (range)	56 (16–63)
Sex (males/females)	14/10
Median initial performance status (range)*	2 (0–3)
No. of patients with prior therapy	
None	17
Radiation	6
Chemotherapy	2

*Cancer and Leukemia Group B criteria.

TABLE 2.—Response by histology

Histology	No. of patients treated	No. of patients with — Response (CR + PR)	No. of patients with — Improvement
Leiomyosarcoma	10	5	2
Mesothelioma	3	0	2
Rhabdomyosarcoma	1	0	0
Cystosarcoma phylloides	2	0	1
Ewing's sarcoma	1	0	1
Osteogenic sarcoma	1	0	0
Synovial sarcoma	1	1	0
Hemangioendothelioma	1	0	1
Malignant fibrous histiocytoma	1	0	1
Totals	21	6 (28%)	8 (39%)

durations of 35 and 47 weeks. Those obtaining PR had 25-, 39-, 63-, and 31-week response durations, and those with disease stabilization had a 24-week median response duration. Four of six previously treated patients had objective regressions to this therapy, with three disease stabilizations and one PR. Median durations of improvement were similar to those of previously untreated patients. Sites of response included liver metastases, lung metastases, primary sites in retroperitoneum and pelvis and mesenteric metastases among five patients with leiomyosarcomas, and liver metastases and a leg primary tumor in one patient with a synovial sarcoma.

The median duration of survival for the entire group of patients was 37 weeks. Patients who responded had a median survival of 50 weeks while those not responding had a median survival of 17 weeks ($P = 0.02$). The two patients with CR survived for 47 and 101 weeks. Median survival times of those patients obtaining PR and disease stabilization were 61 and 41 weeks, respectively. Actuarial survival curves are shown in figure 1.

Drug toxicity seen with this combination is shown in table 3. Moderate granulocytopenia (wbc count $< 3.0 \times 10^3/\mu l$) occurred commonly and was associated with two infectious episodes requiring hospitalization for antibiotic therapy. The dose of doxorubicin was reduced in 36% of courses administered; however, the reductions were for hepatic dysfunction (21%), mucositis (11%), and severe nausea and vomiting (4%), rather than for myelosuppression. Dactinomycin doses were reduced in 17% of courses secondary to toxicity (10% for mucositis, 7% for gastrointestinal toxicity, and none for myelosuppression). Six patients were initially treated with 200 $\mu g/m^2$, but doses were increased to 250 or 300 $\mu g/m^2$ in three patients when mild myelosuppression or no myelosuppression was noted.

Five cases of congestive heart failure occurred among seven patients who received a total dose of 540 mg/m^2 of doxorubicin. These patients ranged in age from 30 to 63 years (median, 59). None had had prior cardiovascular disease, and none had received prior radiation therapy to the mediastinum or chest. Three of these five patients died of heart failure; the other two died of advanced tumor with active heart failure. Congestive heart failure occurred from 1 to 6 months (median, 2.5) following the final dose of doxorubicin. No tissue data were available to confirm the pathologic etiology of these cases of congestive heart failure. Twelve of 24 treated patients developed mucositis; two of these were classified as mild, four as moderate, and six as severe.

PHARMACOKINETICS

The mean terminal half-life of doxorubicin in the four patients evaluated by pharmacokinetic assay was 27.6 hours, and the mean C × T, as determined by the area under the curve, was 11.01 nM/ml × hours. These data correspond to previously performed doxorubicin kinetic studies (13). There was no apparent drug-drug interaction kinetically detectable between doxorubicin and dactinomycin. When the kinetics of the two patients with cardiomyopathy were compared to those of two patients without cardiac difficulties, there was no kinetic difference.

DISCUSSION

This study was initiated to evaluate the possibility of enhancing response rates and durations of response in patients with advanced sarcomas. Both doxorubicin and dactinomycin are active single agents; thus their combination is logical. Most of

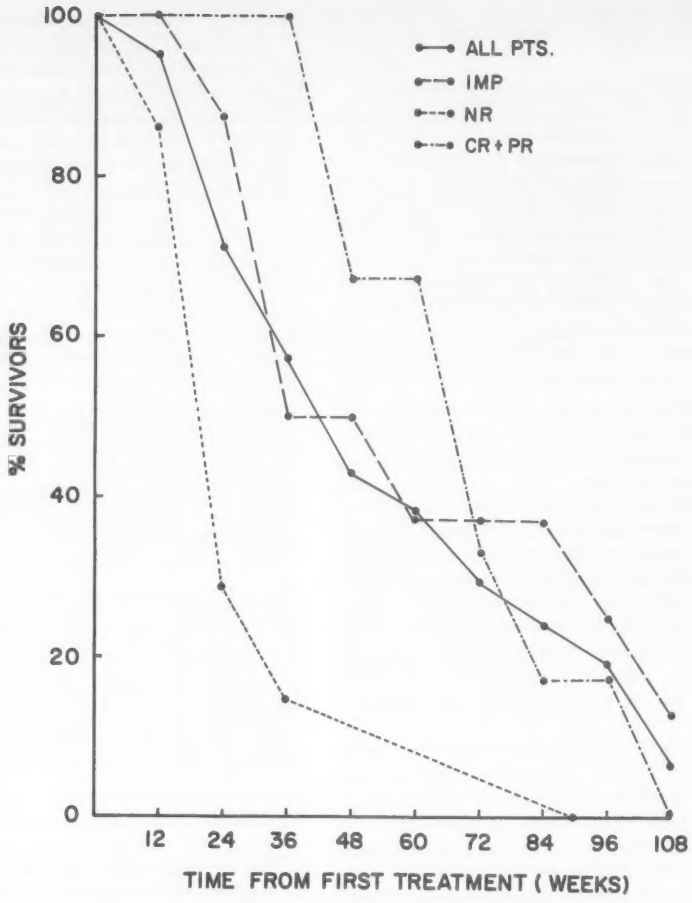

FIGURE 1.—Survival of sarcoma patients receiving doxorubicin and dactinomycin.

the activity of dactinomycin has been demonstrated in childhood sarcomas, particularly rhabdomyosarcoma. Previous studies (1,2,5,15–22) using doxorubicin in the treatment of soft tissue sarcomas have yielded overall response rates of 25%–42% (CR plus PR). Many of these studies (and successes) have been in children with pediatric malignancies.

Sarcomas tend to be uncommon tumors, so that most series combine a variety of tissue types. When combined data are analyzed, patients with certain sarcomas, such as rhabdomyosarcomas, angiosarcomas, leiomyosarcomas, and liposarcomas, tend to obtain better response rates than others with sarcomas such as chondrosarcomas, myxosarcomas, and fibrous histiocytomas (5). In our study, five of six responses were in patients with leiomyosarcomas. While ten of 21 entered patients had leiomyosarcomas, the response rate (50%) still indicates improved responsiveness in this group.

In the current study, patients receiving the combination of doxorubicin and dactinomycin obtained a response rate of 28%. Durations of response and survival times are similar to those reported for doxorubicin alone or to those reported for the combination of doxorubicin and DTIC (1,5,23). As in other studies, those patients obtaining objective responses had improved survival times over the nonresponders, and patients with objective improve-

TABLE 3.—Toxic effects in patients receiving doxorubicin and dactinomycin

Toxic effect	% of patients
Nausea and vomiting	100
Alopecia	100
Mucositis	50
Leukopenia (wbc count $< 3.0 \times 10^3/\mu l$)	35*
Thrombocytopenia (platelet count $< 100{,}000/\mu l$)	12*
Cardiac	21

*Expressed as % of total No. of courses administered.

ments tended to have intermediate survival times (fig 1).

Recently reported multi-agent chemotherapy trials with cyclophosphamide, vincristine, doxorubicin, and DTIC (CYVADIC) have revealed a response rate of 35% at 12 weeks of therapy. These data were not statistically significant compared with an alternate arm of cyclophosphamide and DTIC with a response rate of 23% (3). A recent Southwest Oncology Group study comparing doxorubicin and DTIC (ADIC); cyclophosphamide, doxorubicin, and DTIC (CYADIC); and doxorubicin, DTIC, and dactinomycin found no significant differences among the response rates of 22%, 35%, and 25%, respectively. Survival durations of responders were slightly < 1 year (4). These response rates and durations of survival do not appear to differ from cumulative data of doxorubicin alone reported by Chang (1) or from the current study. A large, recently published study of CYVADIC has revealed a response rate of 50% and a median response duration of 1 year, thus creating confusion as to the true efficacy of this combination (24). Resolution of this conflict will require a large-scale randomized trial of doxorubicin alone versus CYVADIC.

The combination of doxorubicin and dactinomycin did produce increased toxicity. There was a higher incidence of leukopenia and mucositis than was seen in previous studies. Of particular concern was the increased occurrence of congestive heart failure in patients receiving a total cumulative dose of doxorubicin < 550 mg/m². A recent data review suggests that 5% of patients receiving < 550 mg/m² developed congestive heart failure (25); yet in our series, 21% of patients developed this problem. Previous reports (26–29) have noted more frequent development of cardiomyopathy in patients treated with doxorubicin and prior mediastinal irradiation (26), mitomycin C (27), the combination of dactinomycin and mithramycin (28), and DTIC (29). Patients treated with mitomycin C (27) and dactinomycin and mithramycin (28) received these drugs following discontinuation of treatment with doxorubicin and had a higher incidence of cardiomyopathy which was attributed to sequential toxic synergism of these drugs and doxorubicin. In the case of DTIC (29), children were treated simultaneously with doxorubicin and DTIC. Other than doxorubicin, none of the above-mentioned drugs are known causes of cardiomyopathy when used as primary single agents. Although there was no pathologic confirmation of doxorubicin-type cardiomyopathy in our patients, four of five patients developing congestive failure had no previous history of cardiac problems, prior mediastinal irradiation, or acute cardiac events during their clinical courses. Periodic electrocardiograms performed during therapy were not revealing, and no other prospective cardiologic evaluations were performed. The limited pharmacokinetic studies do not explain the increased cardiotoxicity. The possibility may exist that dactinomycin is also toxic to myocardial cells, that it potentiates doxorubicin-induced cardiotoxicity, or both.

In conclusion, the combination of doxorubicin and dactinomycin in advanced sarcomas provides no advantage for the induction of responses, improved response durations, or survival of responders over doxorubicin alone. Recent trials have failed to show superiority of ADIC or CYVADIC over single anthracycline therapy (3,4), and one can question whether any combination available today offers advantage over doxorubicin alone for patients with advanced sarcomas. The marked increase in toxicity seen using our combination makes it less desirable than other established treatments for advanced sarcomas.

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Phase I Study of Pyrazofurin and 5-Azacytidine in Refractory Adult Acute Leukemia[1]

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Writing for the Southeastern Cancer Study Group

SUMMARY

Twenty-eight patients with refractory adult acute leukemia were treated with pyrazofurin and 5-azacytidine. Seven patients showed a response. Dermatitis and mucositis were severe at doses of pyrazofurin > 50 mg/m²/day and precluded administration of multiple courses in most patients. As a result of this toxicity, 16 patients received only one course of chemotherapy. The response rates achieved with this combination were not superior to those achieved with 5-azacytidine alone.

[Cancer Treat Rep 65:237–239, 1981]

Pyrazofurin (PF), a new antipyrimidine drug, and 5-azacytidine (5-AZA) have been shown to have antileukemic effects (1–4). Recently, it was demonstrated that exposure of human and murine leukemic cells to PF resulted in marked accumulation of 5-AZA in these cells (5). In view of these synergistic antileukemic effects, the Southeastern Cancer Study Group began a phase I study of PF and 5-AZA in 1977 in patients with refractory acute leukemia. The results in 28 patients entered in this study form the basis of this report.

MATERIALS AND METHODS

Criteria for patient selection.—Patients entered in this study had to have a diagnosis of adult acute leukemia, myeloblastic or lymphoblastic, that was refractory to previous chemotherapy. Patients signing an informed consent statement had a physical examination, determination of performance status, and other pretreatment tests as follows: bone marrow examination, cbcs, alkaline phosphatase, SGOT, serum protein, BUN, and creatinine. During treatment, cbcs were obtained twice per week, and a bone marrow examination was done every 2 weeks unless leukemic cells were present in peripheral blood.

Doses and schedules.—The initial treatment plan consisted of PF (150 mg/m² by rapid injection) followed 6 hours later by 5-AZA (150 mg/m² by continuous infusion for 5 days). These doses were selected from previous phase I studies for 5-AZA (1,4) and PF (6). Courses of such treatment were to be repeated on Days 15 and 29. Patients were evaluated after three courses of treatment. Response to therapy was judged by criteria previously reported (7). Patients in complete remission (CR) or partial remission (PR) continued receiving treatment to relapse. Patients who had no response (NR) were taken out of the study.

Toxicity evaluation.—Mucositis was graded as follows: *grade 1*—mild mucositis lasting 1–3 days; *grade 2*—moderate mucositis with ulcerations lasting 3–6 days; and *grade 3*—severe mucositis with extensive ulcerations lasting 5 days. Gastrointestinal toxicity was graded as mild, moderate, or severe, depending on the degree of nausea and vomiting and the occurrence of diarrhea. Hepatic toxicity was graded as follows: *grade 1*—an increase of 50% in SGOT or alkaline phosphatase; and *grade 2*—an increase of 100% not attributable to disease. An

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TABLE 1.—Antileukemic effects of PF and 5-AZA treatment

PF dose (mg/m²)*	No. of patients treated	No. of patients with—		
		CR	PR	NR
150	7	0	1	6
100	13	1	2	10
50	4	1	1	2
25	4	1	0	3
Totals	28	3	4	21

*All patients received 150 mg/m² of 5-AZA × 5 days.

TABLE 2.—Toxicity of the PF and 5-AZA combination

PF dose (mg/m²)*	No. of patients treated	No. of patients with toxic effects in—					
		Mucous membranes and skin (grade)			Gastrointestinal tract (grade)†		
		1	2	3	1	2	3
150	7	0	2	5	1	4	2
100	13	1	6	5	8	3	1
50	4	3	2	0	3	2	0
25	4	3	1	0	2	2	0

*All patients received 150 mg/m² of 5-AZA × 5 days.
†1 = mild, 2 = moderate, 3 = severe.

increase to above normal in creatinine or BUN or a decrease in creatinine clearance to 60 ml/minute was considered evidence of renal toxicity. Skin toxicity was graded as follows: *grade 1*—mild asymptomatic nonconfluent erythema; *grade 2*—confluent rash in areas, mildly symptomatic, minimal scaling; and *grade 3*—generalized, painful, weeping lesions with scaling.

RESULTS

Twenty-eight patients were entered in the study. One patient had acute lymphoblastic leukemia, 22 had acute myeloblastic leukemia, and three had acute promyelocytic leukemia. Two patients had acute myelomonocytic leukemia. The ages of the patients ranged from 19 to 70 years. Nineteen patients were < 50 years of age; nine were > 50 years of age. All patients had previously received chemotherapy. Prior therapy for patients with acute myeloblastic leukemia consisted of cytarabine in combination with doxorubicin, daunorubicin, or 6-thioguanine.

The results of treatment with the combination of PF and 5-AZA are summarized in table 1. All patients received 5-AZA at a dose of 150 mg/m² for 5 consecutive days. However, due to severe skin and mucous membrane toxicity, decreasing doses of PF became necessary. PF was decreased stepwise to 25 mg/m² from an initial dose of 150 mg/m².

Overall, of the 28 patients entered in the study, three achieved CR, four achieved PR, and 21 had NR. The mean duration of remission was 3 months.

Toxicity of the PF and 5-AZA combination is shown in table 2. At PF doses of 150 mg/m², skin and mucous membrane toxicity was severe. All patients receiving this dose exhibited diffuse, patchy, cutaneous erythema and edema with severe pruritus, and six patients had oral ulcerations. At lower doses of PF, skin and mucous membrane toxicity was less but was still a severe problem.

Due to severe skin and mucous membrane toxicity, only one course of the combination chemotherapy was possible in 20 of the 28 patients in the following PF dose schedules: five of seven patients given the 150-mg/m² dose, ten of 13 given the 100-mg/m² dose, two of four given the 50-mg/m² dose, and three of four given the 25-mg/m² dose.

The use of prednisone (60 mg/day) decreased the severity of the toxic reactions of the skin and mucous membranes but did not prevent development of skin rash or mucositis, even with lower doses of PF. Of the 21 patients with NR, 16 received only one course of chemotherapy as a result of severe skin and mucous membrane toxic reactions.

DISCUSSION

PF, an inhibitor of pyrimidine synthesis, reduces intracellular levels of pyrimidine ribonucleotides, which in turn results in an accumulation of 5-AZA in murine and human leukemic cells (5). Enhanced phosphorylation of 5-AZA results in increased quantities of 5-AZA-CTP, which in turn inhibits RNA and protein synthesis. These kinetic observations support the use of the combination of PF and 5-AZA in acute refractory leukemia. Normal uptake and phosphorylation of 5-AZA would also be expected to occur in rapidly dividing cells other than bone marrow tissue. Thus, cells undergoing rapid nucleic acid synthesis, such as those in skin and mucous membranes, might also be affected by this combination.

In other studies the use of PF has been noted to produce toxic effects on mucous membranes and skin in approximately 50% of the cases (6). Most reports dealing with toxicity of 5-AZA alone do not report adverse skin reactions. However, Karon et al (8) state that approximately 50% of patients in their series had transient pruritic skin rashes.

In this study PF and AZA appeared to have additive toxic effects on skin and mucous membranes at PF doses > 50 mg/m^2. This toxicity precluded use of PF at higher doses, which may be important for enhanced uptake of 5-AZA by leukemic cells exposed to PF.

In conclusion, the use of PF and 5-AZA in 28 patients with refractory adult acute leukemia produced a response rate of 25%. Toxicity was severe to skin and mucous membranes at PF doses > 50 mg/m^2.

The results of this study indicate a response rate for the combination which is similar to that for 5-AZA alone. We cannot answer the question of whether the combination might be synergistic at higher doses of PF because of the toxicity at those dose levels.

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Treatment of Ewing's Sarcoma With High-Dose Melphalan and Autologous Bone Marrow Transplantation[1]

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SUMMARY

Three patients with disseminated, relapsed Ewing's sarcoma refractory to conventional chemotherapy were treated with high-dose melphalan, cyclophosphamide pretreatment, and bone marrow autotransplantation. Two patients achieved complete remission and one achieved partial remission. The two patients who achieved complete remission remain disease-free at 12 and 13 months. High-dose melphalan is effective when conventional approaches have failed.

[Cancer Treat Rep 65:241–244, 1981]

High doses of melphalan (> 100 mg/m²) cause profound and prolonged bone marrow hypoplasia. It has previously been shown that the period of hypoplasia can be shortened to a clinically acceptable time by autologous bone marrow grafting (1) and by cyclophosphamide pretreatment (2). Early experience with high-dose melphalan in neuroblastoma (3) proved encouraging and raised the question as to whether other childhood tumors could be treated this way. We have therefore used high-dose melphalan to treat three patients with metastatic Ewing's sarcoma refractory to conventional therapy, using the techniques previously reported to minimize normal tissue toxicity. These patients form part of a larger phase I–II study in which the role of high-dose melphalan is being evaluated in solid tumors.

It is important here to stress that the treatment is toxic and should only be given in centers skilled in the administration of cancer chemotherapy and with a high level of patient support facilities. Full details of treatment techniques are given in the paper by McElwain et al (1) which should be read by anyone thinking of giving this treatment. Acute toxic effects include nausea and vomiting, and all patients experience anorexia, epilation, and some soreness of the buccal mucosa during the period of neutropenia.

CASE REPORTS

Patient 1

A 15-year-old boy had Ewing's sarcoma of the right tibia diagnosed in November 1977. There was no evidence of distant metastasis. He was treated with local radiotherapy (right leg and right knee joint) at a dose of 3785 rads in 19 fractions over 29 days with cobalt 60, followed by a dose of 3015 rads in 14 fractions over 22 days with an 8-MeV linear accelerator.

Adjuvant chemotherapy with vincristine (1.5 mg/m²), cyclophosphamide (300 mg/m²), and doxorubicin (40 mg/m²) in combination every 2 weeks was started after the first 19 fractions of radiotherapy. After six courses, dactinomycin (0.6 mg/m²) was substituted for the doxorubicin. In November 1978,

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after a history of chest pain, multiple metastases in both lung fields were seen on whole-lung tomographs. There was no evidence of metastases elsewhere. Whole-lung irradiation (2300 rads in 16 fractions over 30 days with an 8-MeV linear accelerator) together with dactinomycin treatment for the first 5 days (10 μg/kg) was begun, and this was followed with cyclophosphamide (20 mg/kg) at the completion of radiotherapy. A further course of dactinomycin and cyclophosphamide was given after a 2-week interval. There was only a partial response to this therapy, and multiple lung metastases remained visible on whole-lung tomography. The patient was restaged and multiple bone marrow trephines were taken. Disease was confined to the lung. A Hickman catheter was inserted and cyclophosphamide priming at a dose of 500 mg iv was given; 1 week later, melphalan at a dose of 180 mg (120 mg/m^2) was administered immediately after bone marrow harvest. This was a lower dose than the 140 mg/m^2 previously given to other patients (1,3). The dose reduction was made in view of the close proximity of the extensive previous treatment with chemotherapy and radiotherapy and its possible cumulative toxicity to the bone marrow. The bone marrow was stored heparinized at 4°C and was infused 8 hours after the melphalan injection (1). The total number of nucleated cells infused was 1.4×10^{10} (2.7×10^8/kg).

The patient had significant neutropenia (neutrophil count $< 0.8 \times 10^9$/liter) on Day 10 after the melphalan and significant thrombocytopenia (platelet count $< 50 \times 10^9$/liter) on Day 12. Neutrophil count recovery occurred on Day 19 and platelet count recovery occurred on Day 24. Repeat tomography showed complete regression of lung metastases, aside from one nodule (0.5 cm) in one cut. This was confirmed by a computerized tomographic (CT) scan and was resolved after a further course of melphalan given at a lower dose (50 mg/m^2) with autologous grafting and cyclophosphamide priming. Significant neutropenia occurred on Day 14 with recovery on Day 23, and significant thrombocytopenia occurred on Day 11 with recovery on Day 26. A third course of melphalan with further dose reduction was given (35 mg/m^2) with autologous grafting and cyclophosphamide priming. These dose reductions were again made because of the possibility of irreversible bone marrow damage from the previous treatment. Significant neutropenia did not occur (lowest neutrophil count, 1.5×10^9/liter). The platelet count was $< 50 \times 10^9$/liter for 2 days (Days 23 and 24). Subsequently, there was full recovery of the peripheral blood cell count. The patient remains in complete remission at 13 months (May 1980). Hematologic data for this patient and the other two patients are shown in table 1.

Patient 2

A 16-year-old girl was found to have Ewing's sarcoma confined to the sacrum in June 1976.

Incomplete surgical excision was followed by radiotherapy at a dose of 4600 rads in 25 fractions over 42 days with cobalt 60, followed by a dose of 1750 rads in seven fractions over 7 days with 34 MeV electrons. At the same time, adjuvant chemotherapy with vincristine (1.5 mg/m^2), doxorubicin (40 mg/m^2), and cyclophosphamide (300 mg/m^2) was begun. After six courses, dactinomycin (0.6 mg/m^2) was substituted for doxorubicin, and chemotherapy was continued at monthly intervals from November 1976 to June 1978. In July 1978, an opacity was noted in the left lower lobe on chest roentgenogram and comprehensive restaging was undertaken. The metastasis was solitary and in November 1978 it was removed surgically. She remained well until May 1979 when a further lung metastasis was found and CT scanning of the lungs revealed the presence of six deposits 1.5–2 cm in diameter. All other investigations, including bone scan, bone marrow aspirate, and trephine were normal. One week after cyclophosphamide (500 mg iv) she received a dose of 390 mg of melphalan iv (215 mg/m^2) in conjunction with autologous marrow rescue (1.47×10^{10} cells harvested in a total volume of 990 ml, equivalent to 2.2×10^8 nucleated cells/kg of body weight). It was felt that a higher dose of melphalan could be given than that given to patient 1 since patient 2 had received no chemotherapy for 11 months.

Significant neutropenia (neutrophil count $< 0.8 \times 10^9$/liter) developed on Day 6 and persisted until Day 15. Thrombocytopenia (platelet count $< 50 \times 10^9$/liter) developed on Day 7 and persisted until Day 18. Support in the form of iv antibiotics and platelet transfusions were given as necessary. Wbc transfusions were not required.

With complete resolution of the chest roentgenogram lesions, a second course of melphalan at a slightly lower dose (200 mg/m^2) was given 3 months after the first treatment. The patient received a total dose of 340 mg, again in conjunction with cyclophosphamide pretreatment and autologous marrow rescue (1.24×10^{10} cells harvested in 764 ml marrow, equivalent to 2×10^8 nucleated cells/kg of body weight).

Hematologic recovery was substantially slower, neutropenia persisted from Day 3 to 21, and the platelet count remained at around 20×10^9 liter.

TABLE 1.—Drug dose and hematologic data

Patient No.	Course No.	Melphalan dose (mg/m^2)	Days patient had—	
			Neutropenia	Thrombocytopenia
1	1	120 (180 mg)	10–19	12–24
	2	50 (80 mg)	14–21	11–26
	3	35 (50 mg)	—	23–24
2	1	215 (390 mg)	6–15	7–18
	2	200 (340 mg)	3–21	6–42
3	1	200 (300 mg)	6–25	9–40

As of May 1980, complete remission (confirmed by CT scan) has been maintained for 12 months, and the patient is living and working normally. Her platelet count is 80 × 10^9/liter.

Patient 3

A 20-year-old man was referred with disseminated Ewing's sarcoma diagnosed 7 years previously. In 1972 he had been found to have Ewing's sarcoma confined to the sacrum. He was treated with radiotherapy alone (4000 rads in 29 fractions over 42 days with cobalt 60) and remained well until January 1979, when a rapidly enlarging metastasis was noted in the scalp, with a similar deposit in the right mandible. The liver was enlarged 15 cm below the costal margin.

Chest roentgenogram showed tumor at the left hilum, bone scan showed widespread metastatic disease, and liver scan showed massive filling defects. Bone marrow aspirate and trephine showed no evidence of infiltration.

He was treated initially with vincristine (1.4 mg/m^2), doxorubicin (30 mg/m^2), and cyclophosphamide (600 mg/m^2) every 2 weeks with a rapid regression of disease at all sites. However, after nine courses (total dose of doxorubicin, 450 mg) the scalp lesion, lung metastases, and liver were again found to be increasing in size. Marrow aspirate and trephine were again clear of tumor and he was then treated with melphalan 1 week after pretreatment with cyclophosphamide (500 mg iv). He received melphalan at a dose of 200 mg/m^2 (total dose, 300 mg) with autologous marrow rescue.

Routine inspection of the harvested marrow revealed one of the two bags to be heavily infiltrated with tumor cells, so that only the second bag (tumor-free on all of the eight films examined) was returned to him 8 hours after the melphalan, containing 0.5 × 10^{10} nucleated cells (1.02 × 10^8/kg of body weight).

Hematologic recovery was relatively slow, with neutropenia (neutrophil count < 0.8 × 10^9/liter) persisting from Day 6 to 25 and thrombocytopenia (platelet count < 50 × 10^9/liter) persisting from Day 9 to 40. Routine supportive measures (iv antibiotics, platelet transfusions, etc) were used as previously described (1). Wbc transfusions were not required. There was early and dramatic relief of bone pain, which had previously required regular opiate analgesia. In addition, there was regression of lung and liver metastases, and complete regression of the scalp metastasis. (His liver, which had measured 15 cm below the costal margin, regressed until it was impalpable.) The sites at which bone marrow infiltration had been found were biopsied and found to be tumor-free.

This response was incomplete and short-lived; within 3 months there was again evidence of disease progression at all sites. For this reason it was felt that there was no indication to re-treat with melphalan and the patient died 3 months later.

DISCUSSION

Ewing's sarcoma has been shown to be sensitive to a wide variety of cytotoxic drugs, and the introduction of adjuvant chemotherapy (with vincristine [4], cyclophosphamide [5], dactinomycin [6], and doxorubicin [7]) has led to a marked increase in disease-free survival. Similar combinations have been used successfully in the treatment of established metastatic disease and although an initial response is frequent, relapse rates are high (8). The three patients reported here had widespread metastatic disease which had relapsed after conventional therapy. In spite of previous chemotherapy, all patients tolerated treatment with high-dose melphalan without undue toxicity, although our experience with patient 2 shows that when very high drug doses are given, even with autotransplantation of bone marrow, recovery of the bone marrow is slower after the second treatment than after the first. This finding suggests that the number of courses of high-dose melphalan that could be given to a patient is limited, perhaps to two or three.

Hematologic recovery was predictably slower than that in previously untreated patients (1). This was marked in the two patients who had a second course but presented no significant difficulty. Two of the three patients achieved a complete remission and the other achieved a partial remission; it is of particular interest that previous treatment with conventional doses of cyclophosphamide did not abolish the sensitivity of the tumors to high-dose treatment with a different alkylating agent.

We do not know how long these remissions will last, but the possibilities for incorporating very intensive chemotherapy into the overall management of patients with Ewing's sarcoma clearly need consideration. For example, the late sequelae of intensive radiation therapy together with a failure to control micrometastatic disease have led Rosen et al (8) to propose an "early intensification" of chemotherapy, with a seven-drug cycle (containing vincristine, dactinomycin, cyclophosphamide, bleomycin, doxorubicin, BCNU, and methotrexate) given before radiotherapy and/or surgery. The use of high-dose melphalan with autograft may be an equally appropriate approach or alternatively it might be used as a form of "late intensification" at the end of a course of conventional adjuvant chemotherapy. This second plan is currently under investigation in our Units in patients with Ewing's sarcoma and metastatic neuroblastoma.

ADDENDUM

Two further patients, 4 and 11 years of age with disseminated Ewing's sarcoma resistant to conventional chemotherapy, were treated with melphalan (200 mg/m^2) and autologous bone marrow; both achieved complete remission after one treatment.

In four of five patients who achieved complete remission, three have relapsed (two at 14 months and one at 5 months) and the fourth remains in remission at 3 months.

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Vindesine in the Treatment of Small Cell Anaplastic Bronchogenic Carcinoma[1,2,3]

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SUMMARY

The antineoplastic activity of vindesine was evaluated in a phase II trial in patients with small cell anaplastic bronchogenic carcinoma. All patients had been treated previously with vincristine, CCNU, cyclophosphamide, VP-16-213, methotrexate, and doxorubicin. In some of the patients, the primary tumor had also been irradiated, and some patients had received 5-FU or procarbazine. The dose of vindesine was 4 mg/m²/week iv, modified according to hematologic and neurologic side effects. Twenty-six of 32 patients included in the trial were evaluable. Objective response was observed in seven patients (27%). The median duration of response was 35 days (range, 28–90), the response occurring within 14 days after initiation of treatment. Dose modification of vindesine was necessary in 80% of the patients because of hematologic toxicity, while neurotoxicity made dose reductions necessary in about one half of the patients. The study demonstrates that vindesine is active against small cell bronchogenic carcinoma, with apparent lack of clinical cross-resistance to vincristine in previously heavily treated patients.

[Cancer Treat Rep 65:245–248, 1981]

Within the last few years, the new semisynthetic vinca alkaloid vindesine has been clinically evaluated in a variety of malignant disorders. Generally, vindesine has been found to be active in neoplasms sensitive to either vincristine or vinblastine. No activity has been found in generally resistant tumors, such as colorectal (1) or renal cell (2) cancers. In previously treated patients, response rates of 10%–20% have been obtained in testicular germ cell cancer (3) and Hodgkin's and non-Hodgkin's lymphomas (4). In studies including some previously untreated patients, response rates of 30% in breast cancer (5) and 50%–60% in acute lymphatic leukemia and blastic crisis of chronic myeloid leukemia (6) have been obtained. The additional observation that patients previously treated with either vincristine or vinblastine responded to vindesine possibly indicates a lack of clinical cross-resistance between these vinca alkaloids.

In patients with lung cancer, vindesine has been described as having a relatively high activity with a response rate of > 20% in the combined group of adenocarcinoma and large cell and epidermoid cell carcinomas (7), in contrast to the relatively poor results of vincristine therapy in these diseases. Small cell bronchogenic carcinoma is, however, highly sensitive to vincristine (8), so we found it relevant to examine the activity of vindesine against this neoplasm.

MATERIALS AND METHODS

All patients were eligible for the study who had histologically proven small cell anaplastic bronchogenic carcinoma, with progressive disease in spite

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[3]Vindesine was supplied by Eli Lilly and Co, Indianapolis, IN.

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TABLE 1.—Dose modifications of vindesine

Dose (%)	Toxic effects	
	*Wbc count**	*Platelet count**
100	> 4000	> 100,000
67	> 3000–4000	75,000–100,000
33	> 2000–3000	50,000–74,999
0	< 2000	< 50,000
50	Constipation (> 3 days)	
50	Pain (abdominal/jaw)	
50	Paresthesias (socially disabling)	
50	Weakness (walking distance < 30 m)	
25	Continued neurotoxicity in spite of dose reduction to 50%	
0	Continued neurotoxicity in spite of dose reduction to 25%	

*Cells/mm^3.

of intensive combination chemotherapy in regimens including vincristine.

Treatment with vindesine was not initiated until hematologic restitution after prior therapy. A wbc count of ≥ 4000/mm^3 and a platelet count of ≥ 100,000/mm^3 were thus awaited unless bone marrow invasion could be or had previously been bioptically proven.

Criteria for inclusion were measurable or evaluable disease with an expected survival of at least 4 weeks. Clinical examinations were performed before the start of therapy, and every 2 weeks thereafter. Cbcs were performed every week. Serum electrolytes, creatinine, alkaline phosphatase, lactate dehydrogenase, and bilirubin, prothrombin time, and chest roentgenograms were performed every 4 weeks.

The dose of vindesine was 4 mg/m^2/week iv; it was modified according to side effects as shown in table 1. Treatment was continued until progression of disease.

Objective regression was defined as at least a 50% reduction of the square product of two perpendicular diameters of a parameter or a 75% reduction in lesions which for technical reasons could not be measured exactly. The duration of the response was counted from detection of disease until progression. Disease progression was defined as a > 25% increase of a parameter or the appearance of new metastases. The occurrence of CNS metastases was not considered as disease progression.

In order to be evaluable, patients with clinically measurable disease should be treated for at least 2 weeks, while patients with only a radiologic lesion should be treated for 4 weeks.

All patients had given informed consent and had accepted the investigational character of the treatment.

Differences between groups of unpaired data of this study were tested for statistical significance by the nonparametric Mann-Whitney rank-sum test.

RESULTS

Thirty-two patients were included in the trial. All patients had previously been treated in a phase III trial with cyclophosphamide, CCNU, methotrexate, VP-16-213, doxorubicin, and vincristine. Five patients had subsequently been treated with 5-FU, and two had been treated with procarbazine. In eight patients, the primary tumor and the mediastinum had been irradiated (40 Grey).

Three of the 32 patients were not evaluable because they died from rapid thoracic tumor progression 2, 4, and 9 days after initiation of vindesine therapy. One patient died on Day 18 because of brain metastases. The measurable lesion was the lung tumor, so the treatment result was not evaluable. One patient refused both additional treatment and control in the department and was thus inevaluable. Irradiation was necessary in one patient with superior vena cava syndrome 8 days after initiation of vindesine therapy. He responded to the combined treatment but was regarded as having disease progression, so 26 patients were left for therapeutic evaluation. The median age of these patients was 58 years (range, 38–67).

No complete response was observed, but partial responses were obtained in seven patients, resulting in a response rate of 27% (range, 12%–47%; 95% confidence limits). The median duration of response was 35 days (range, 28–50).

In five patients, the responses were observed in the primary tumor, and in two patients they were observed in metastatic lesions.

No major differences in extent or distribution of disease were observed between responding and nonresponding patients. The average performance status of responders was 1.3 compared to 1.7 for the entire group (Eastern Cooperative Oncology Group performance scale).[5]

Patients responding to vindesine were distributed proportionately between the various combinations of previous therapy. Also, the duration of treatment prior to vindesine was almost the same for responders and nonresponders (medians of 340

[5]Manual for Staging of Cancer. Prepared by the American Joint Committee for Cancer Staging and End-Result Reporting, Chicago, 1977, p 5.

TABLE 2.—Hematoligic toxicity

	No. of patients* (%)
Hemoglobin nadir (mmols/liter)†	
< 7	24 (91)
< 6	5 (19)
< 5	1 (4)
Wbc count nadir (cells/mm³)	
< 4000	26 (100)
< 3000	21 (79)
< 2000	13 (50)
< 1000	4 (17)
Platelet count nadir (cells/mm³)	
< 100,000	4 (17)
< 80,000	1 (4)

*No. of patients experiencing a blood cell count nadir below the levels shown.
†7 mmols/liter = 13.5 g/dl.

[range, 85–501] and 320 [range, 73–853] days, respectively [$P > 0.10$]).

No significant difference ($P > 0.10$) was observed in the total amount of VP-16-213 received by responders compared to nonresponders. The median total dose in responders was 1.4 g (range, 1.0–5.2) compared to 2.4 g (range, 0.8–5.6) in nonresponders.

All patients had previously been treated with vincristine. The median cumulative dose of vincristine was 16.5 mg (range, 9–24) for responders and 16 mg (range, 10–33) for nonresponders ($P > 0.10$).

The time from the last dose of vincristine to the first dose of vindesine was the same in responders and nonresponders: 14 (range, 4–20) and 14 weeks (range, 4–45), respectively ($P > 0.10$).

Vindesine was given for a median of 36 days (range, 17–154), and a median of 71% (38%–100%) of the planned dose was given. Due to toxicity, the average total dose declined proportionately to the duration of treatment, but no reverse relationship between the previous dose of vincristine and that of vindesine was observed.

The dose-limiting toxicity was mainly hematologic (table 2). Thus, doses were reduced in one or more cycles for all but one patient, and 20 of 148 non-first cycles had to be deleted due to leukopenia. In two patients, discontinuation for 2 and 3 weeks was also necessary due to leukopenia. Five patients had wbc counts < 1000/mm³, but no cases of septicemia were observed.

Platelet counts < 100,000/mm³ were recorded in only three patients, the lowest nadir being 71,000/mm³.

Anemia was rare. Blood transfusions were only given once in nine patients and twice in two.

The neurologic side effects observed were primarily paresthesias, constipation, and muscular weakness. Twelve treatment cycles in six patients from a total of 148 non-first cycles were deleted because of neurotoxicity. Constipation was generally alleviated by laxatives, but dose reductions were necessary in nine patients. Almost all patients had paresthesias, but dose modifications were necessary in only eight patients. The muscular weakness was insidious and seldom appeared during the first 4–6 weeks of therapy, and no dose reductions were made specifically because of that symptom. Three cases of jaw pain and two cases of abdominal pain required potent analgesics and dose reductions.

In one case, therapy was discontinued when the patient developed desquamating dermatitis of the hands and feet in addition to relatively severe neurotoxicity.

Alopecia and tendon reflexes were not evaluable because of the previous chemotherapy.

DISCUSSION

This study documents that vindesine is active in the treatment of small cell anaplastic bronchogenic carcinoma, with apparent lack of cross-resistance to vincristine. The observed response rate and duration of response were inferior to the results with vincristine in a previous phase II trial performed at this institution (8).

However, 50% of the patients in the latter trial were previously untreated, and none of the remaining patients had received vinca alkaloids.

The response to the previous chemotherapy in the seven patients responding to vindesine had been

complete in one patient and partial in three, while the last three had disease progression or stable disease. Accordingly, the tumors of the last three patients must have been primarily resistant to vincristine in the dose and schedule used. The four previously responding patients might theoretically have obtained new remissions if the dose of vincristine had been continued on a weekly schedule, even though it seems unlikely because the drug had already been given in a dose causing neurotoxicity.

The therapeutic effect of vinca alkaloids has been observed to increase with the duration of exposure both in experimental animal tumors (9) and in human breast cancer.[6] Our patients, however, received both vincristine and vindesine as bolus injections, so this mechanism does not explain the observed activity of vindesine in this trial.

Clinical lack of cross-resistance between vindesine and vincristine, as in this study, and between vindesine and vinblastine has also been shown in patients with ALL or blast cell crisis of CML with prior treatment failure of a combination of prednisone, L-asparaginase, and vincristine (6) and in patients with germ cell tumors and with Hodgkin's and non-Hodgkin's lymphomas (3,4).

In experimental animal tumors, both antagonism and synergism have been observed between vincristine and VP-16-213, depending on the duration of the interval between the administrations (10). In our patients no such interactions would have been possible during the previous therapy. Cross-resistance between VP-16-213 and vinca alkaloids in human neoplasms has not been described, and the results of this trial are not suggestive of such mechanisms.

In the future, the use of vindesine in the therapy of small cell carcinoma should be clarified, particularly its role in relation to vincristine and vinblastine. First, the efficiency of vinblastine should be examined, however (11); next, the relative therapeutic value of the active vinca alkaloids in combination regimens should be examined; and finally, including alternating administrations of different vinca alkaloids in a combination regimen might delay or prevent appearance of resistance, and less neurotoxicity may make a continued weekly dose possible.

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Phase I Study of ICRF-187 Using a Daily for 3 Days Schedule[1]

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SUMMARY

ICRF-187, the enantiomer of ICRF-159 formulated for iv use, was administered to 23 patients in a phase I clinical trial. The dose ranged from 500 to 1500 mg/m² every day for 3 days and was repeated every 28 days. The toxic effects included moderate to severe leukopenia and thrombocytopenia, which recovered by the 21st day of a treatment cycle. Myelosuppression was more severe in patients with prior nitrosourea treatment. Nonmyelosuppressive toxic effects included nausea and vomiting, transient elevations in liver function tests, alopecia, and increased urinary clearances of iron and zinc. The starting dose for phase II trials should be 1250 mg/m² daily for 3 days repeated at 21-day intervals.

[Cancer Treat Rep 65:249–252, 1981]

ICRF-159 has exhibited antitumor activity in patients with non-Hodgkin's lymphoma (1), colorectal carcinoma (2,3), and Kaposi's sarcoma (4). Early in the clinical trials of ICRF-159 it was noted that oral absorption of the compound was erratic (5). Poor solubility of ICRF-159 as a racemic mixture precluded its parental formulation. Recently, Repta and colleagues (6) found that the (+) enantiomer of ICRF-159, designated ICRF-187 (fig 1), had a solubility which allowed formulation for parenteral use. Its solubility is approximately 10 mg/ml in water whereas that of ICRF-159 is 3 mg/ml in water (6).

Because of clinical activity of the racemic form ICRF-159, the enantiomer ICRF-187 was particularly attractive for clinical trials, as the iv form could provide improved bioavailability and, perhaps, improved antitumor activity.

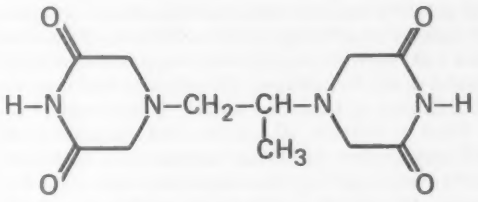

FIGURE 1.—Structure of ICRF-187.

Preclinical toxicology studies of ICRF-187 were performed in beagle dogs. The major clinical symptoms noted in dogs included anorexia, emesis, diarrhea, weight loss, hematemesis, and hematochezia. Leukopenia was common but thrombocytopenia was noted only occasionally. Elevation of hepatic enzymes occurred sporadically. Histologic alterations included bone marrow hypoplasia, necrosis and hemorrhage in the lymphatic and gastrointestinal systems, renal necrosis, testicular degeneration, and cytoplasmic vacuolization in the liver.

MATERIALS AND METHODS

Selection of Patients

Studies with ICRF-187 were initiated in January 1979. All patients entered in the study had histo-

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TABLE 1.—Phase I ICRF-187 study: patient characteristics

Characteristic	No. of patients
Total	23
Men/women	9/14
Age in yrs	
Median: 52	
Range: 5–69	
Prior treatment	23
Chemotherapy	23
Radiotherapy and chemotherapy	9
Tumor type	
Melanoma	7
Sarcoma	6
Acute lymphocytic leukemia	3
Ovarian carcinoma	3
Breast carcinoma	2
Colon carcinoma	1
Lymphoma	1

logic confirmation of a malignancy refractory to standard treatment (table 1). Only patients who had an estimated survival of > 6 weeks were eligible. All patients had received multiple chemotherapeutic agents (an average of five different drugs) and 37% had received prior radiotherapy to the primary tumor or its metastases. All patients had measurable disease at the time of entry in this study.

Prior to therapy, all patients had adequate blood cell counts (wbcs > 4000/mm³ and platelets > 100,000/mm³) except for the three patients with acute leukemia. All patients had normal renal function (BUN < 25 mg/100 ml and serum creatinine ≤ 1.2 mg/100 ml), SGOT < 40 IU/100 ml, alkaline phosphatase < 85 IU/100 ml, and bilirubin ≤ 1.5 mg/100 ml.

Prior to entry in the study, informed consent was obtained from the patient in accordance with federal and institutional policies.

Treatment Plan

ICRF-187 is formulated as a sterile, lyophilized powder. Prior to use each vial containing 250 mg of lyophilized ICRF-187 is reconstituted with 25 ml of sodium chloride, USP. The resulting clear solution contains 10 mg of ICRF-187/ml of fluid. This solution was finally diluted with 150 ml of 5% dextrose in water and was infused through a newly started well-running iv line. The schedule of drug administration was a daily dose for 3 consecutive days. This constituted a course of the drug. A course of treatment was repeated every 28 days. The daily × 3 schedule was chosen because a single dose would require too much fluid administration (> 3 liters because of poor solubility) and because comparative clinical information was available using the daily × 3 schedule for ICRF-159.

Since no animal toxicology data were available for a daily × 3 schedule, the starting dose of 500 mg/m² was determined in the following manner. The toxic dose low for a single-dose schedule in the dog was 10,000 mg/m². One third of this (3333 mg/m²) would be the starting dose in man as a single dose. For safety, the present phase I study began at 500 mg/m² given daily for 3 days. Dose levels were increased as noted in table 2, with a minimum of three patients being treated at each dose level. If no toxicity was observed at a particular dose level after 4 weeks, the next dose level was started. No dose escalations were done in the same patient in an effort to define whether cumulative toxicity would occur with monthly doses of ICRF-187.

Study Parameters

During treatment, the patients had cbcs with differential and platelet counts, SMA-12 chemistry panels, and urinalyses twice per week. In addition, plasma levels and urinary excretion of iron, calcium, zinc, magnesium, and copper were measured before and during treatment with ICRF-187. Other laboratory and radiologic examinations pertinent to tumor evaluations as well as electrocardiograms were performed prior to every course of treatment. Patients were followed closely for any evidence of toxicity and for other biologic effects and were consid-

TABLE 2.—Myelosuppression by ICRF-187 at each dose level

Dose (mg/m², daily × 3)	No. of patients/ No. of courses	Median (cells × 10³/mm³)	
		Wbc count nadir (range)	Platelet count nadir (range)
500	4/7	2.4 (2.2–2.6)	183 (157–209)
750	3*/3	3.0 (2.8–10)	159 (150–175)
1000	9/16	2.5 (0.3*–5.9)	149 (12*–338)
1250	4/4	2.3 (0.4–7.0)	110 (48–300)
1500	3/3	0.7 (0.4–1.2)	49 (0.7–87)

*Patients had prior treatment with a nitrosourea.

ered evaluable for toxicity if they received at least one course (3 days) of ICRF-187 with at least 3 weeks of followup. Standard criteria of response were used to evaluate the antitumor effect of the drug.

Termination of Study

Individual patients were taken off the study if objective tumor progression occurred following one or more courses of ICRF-187. The phase I study was terminated when the maximally tolerated dose was established and when the cumulative toxicity of ICRF-187 was defined.

RESULTS

Over a 10-month period, 23 patients were entered in the study. All patients were evaluable for toxicity. Patient characteristics, including tumor types, are shown in table 1. The sequences of doses and toxic effects observed at each dose level are shown in table 2.

Myelosuppressive Toxicity

Leukopenia and thrombocytopenia were the major dose-limiting toxic effects of ICRF-187 (table 2). A total of 30 courses administered to 20 patients were evaluable for myelosuppression. The three patients with leukemia were not evaluable for myelosuppression. As noted in table 2, the degree of leukopenia was related to the dose of ICRF-187 administered. At the initial dose of 500 mg/m^2 no appreciable leukopenia was noted. At a dose of 1000 mg/m^2 the median wbc count was 2500/mm^3. However, the lowest wbc count (300/mm^3) was seen in a patient previously treated with chlorozotocin. Because of this marked leukopenia, subsequent patients with prior nitrosourea therapy were entered at a lower dose level of 750 mg/m^2. The three patients with prior nitrosourea therapy at that level did not have major leukopenia. Escalation to 1000 mg/m^2 in two additional patients with prior nitrosourea treatment demonstrated wbc count suppression to nadirs of 1200 and 1800/mm^3, respectively. For patients without prior nitrosourea treatment, escalations to doses of 1250 and 1500 mg/m^2 were performed. A dose of 1250 mg/m^2 for 3 days is the maximally tolerated dose in terms of leukopenia in patients without prior nitrosourea treatment. The day of wbc count nadir ranged from Day 8 to Day 15 (median, Day 11) after beginning of therapy, with recovery by Day 21.

The pattern of development of thrombocytopenia has been similar to that of leukopenia. No thrombocytopenia was noted at a dose of 500 mg/m^2, but a platelet count of 12,000/mm^3 was noted in one patient entered at a dose of 1000 mg/m^2 who had had prior nitrosourea therapy. Three patients with prior nitrosourea treatment were entered at a lower dose level (750 mg/m^2) and did not have significant thrombocytopenia. Escalation to a dose of 1000 mg/m^2 in two additional patients with prior nitrosourea treatment resulted in platelet counts of 85,000 and 95,000/mm^3, respectively.

For patients without prior nitrosourea treatment, doses were escalated to 1250 and 1500 mg/m^2. A dose of 1250 mg/m^2 for 3 days was also the maximally tolerated dose in terms of thrombocytopenia. The day of platelet count nadir ranged from Day 9 to Day 15 (median, Day 12) after beginning of therapy, with recovery by Day 21.

Nonhematopoietic Toxicity

All of the 33 courses administered to 23 patients were evaluable for nonhematopoietic toxicity. The toxic effects included elevations in SGOT and SGPT to twice normal (three of 23 patients). These abnormalities peaked on Day 8 after the beginning of administration and were resolved by Day 17. These elevations were felt to be secondary to drug administration. Serum bilirubin and alkaline phosphatase did not increase.

Nausea and vomiting were seen in three patients at higher dose levels but in no instance were these effects severe or dose-limiting. Two patients had partial alopecia. Urinary clearance of iron and zinc increased by an average of tenfold in all five patients studied (table 3). Serum iron increased significantly in four of the five patients while serum zinc decreased significantly in two of the five (204% and 75% of baseline values, respectively). The changes in serum heavy metals did not appear to be related to hepatic toxicity of ICRF-187. These changes were probably caused by the chelating ability of the drug. Total iron binding capacity in the patients' sera was not measured.

Antitumor Activity

No complete or partial remissions were seen in this phase I trial.

DISCUSSION

The present phase I trial suggests that in patients previously treated with nitrosoureas, the dose for phase II studies of ICRF-187 should be 1000 mg/m^2 daily for 3 days. In patients previously treated with

TABLE 3.—Clearance of metals

	Iron	Zinc	Calcium	Magnesium	Copper
Before treatment*	0.025 ± 0.029	0.53 ± 0.31	0.69 ± 0.54	2.25 ± 0.96	0.016 ± 0.008
During ICRF-187*	0.25 ± 0.16	5.35 ± 5.02	1.33 ± 0.59	2.78 ± 1.55	0.28 ± 0.008
During/before ratio	10.0†	10.2‡	1.9	1.2	1.1

*Values = ml/min/1.7 m^2 ± SD.
†$P < 0.01$.
‡$P < 0.05$.

drugs other than the nitrosoureas the starting dose for phase II trials should be 1250 mg/m^2 given daily for 3 days with repeated courses every 21 days.

The dose-limiting toxic effects of ICRF-187 were leukopenia and thrombocytopenia. The myelosuppression was not cumulative. Nonmyelosuppressive toxic effects included mild SGOT and SGPT elevations, occasional vomiting, and occasional alopecia. Also of note were marked increases in urinary clearances of iron and zinc. Serum levels of iron increased while serum zinc levels decreased.

ICRF-187 is theoretically attractive because it essentially represents an iv formulation of the orally administered compound ICRF-159, which did have some antitumor activity in earlier clinical trials (1–4). Although we did not observe any antitumor activity in the extensively previously treated patients in this phase I trial, ICRF-187 should at least have a clinical phase II trial against those tumor types where ICRF-159 activity has been noted, namely, in patients with non-Hodgkin's lymphoma, Kaposi's sarcoma, and previously untreated colorectal carcinoma.

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Plasma Levels and Urinary Excretion of Methyl-GAG Following Iv Infusion in Man[1,2]

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SUMMARY

The pharmacokinetics of methyl-GAG was studied after nephrectomy in five patients with renal cell carcinoma and in one patient with breast cancer. Following rapid infusion (30 minutes) of 700 mg/m² of methyl-GAG, plasma concentrations declined in a biphasic mode with an apparent terminal half-life of 136–224 hours. Drug levels declined to 6%–20% of the initial plasma concentration in the first 24 hours. Approximately 40% of the dose was recovered in the urine after 2 weeks. Extensive extravascular distribution of methyl-GAG is suggested.

[Cancer Treat Rep 65:253–257, 1981]

Methyl-GAG is an antineoplastic agent with activity in acute myelocytic leukemia (1), esophogeal carcinoma (2), breast carcinoma (3), rectal carcinoma (3), malignant lymphoma (3), and renal cell carcinoma. In the early 1960s it underwent intensive clinical trials. Although it was found to have major activity in acute myelocytic leukemia on a daily dose schedule, the therapeutic index was narrow and toxicity was severe and often life-threatening. Toxic effects (1,4,5) included nausea, vomiting, diarrhea, gastrointestinal mucositis, malaise, anorexia, hypoglycemia, hypotension, cutaneous lesions, vasculitis, cellulitis, and, rarely, bilateral peripheral neuropathy. Various alterations in drug dosing schedules were attempted in an effort to improve the therapeutic index. Only the weekly and biweekly (3) schedules appeared promising in that toxicity was acceptable.

Because of the early clinical experience and preliminary pharmacokinetic data in animals (6), which suggested a prolonged half-life, current phase II studies evaluating methyl-GAG in the treatment of a variety of solid tumors involve administration of the drug on a weekly schedule. Toxicity appeared to be acceptable after a single dose; however, after repeated dosing it became more frequent and increased in severity. In a series of 109 patients (7), only 8% experienced side effects following the first dose (500–600 mg/m²), but after two or three additional weekly treatments 50% experienced toxic reactions. Consequently, we attempted to give a larger dose of methyl-GAG every 2 weeks to try to avoid the late toxicity and to obtain additional information regarding drug distribution and elimination. The toxicity seen clinically after repeated weekly doses may be associated with slow excretion and accumulation of the drug; however, a lack of suitable analytic methodology has prevented the evaluation of this postulate. Recently, we described a specific procedure for the analysis of methyl-GAG in plasma and urine (8). The drug is initially separated from the biologic matrix by retention on a small column packed with weak cation exchange resin. After selective elution from the resin, methyl-GAG is quantitated by high-performance liquid chromatography with spectrophotometric detection. The procedure is sensitive and is capable of

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[4]The technical assistance of Mrs. Fong Mei Lee Shih is gratefully acknowledged.

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discriminating between parent drug and any potential metabolites.

In this report we present data obtained from the analysis of the plasma and urine samples of six patients following administration of methyl-GAG by iv infusion. Pharmacokinetic analysis of the data revealed an extremely long terminal half-life with probable extensive distribution of drug to extravascular tissues.

MATERIALS AND METHODS

Patients and Protocol

Six patients received methyl-GAG for treatment of metastatic malignancies after informed consent was obtained according to institutional guidelines. Five patients had renal cell carcinoma with previous nephrectomies, and one had breast cancer. All patients had serum creatinines of ≤ 1.3 mg/100 ml and creatinine clearances of 38–67 ml/minute. Liver function was adequate in all patients as defined by normal serum bilirubin, with SGOT and alkaline phosphatase levels ≤ 100% of normal.

Methyl-GAG was supplied by the National Cancer Institute, Bethesda, MD, and was administered in sterile normal saline solution. Patients were hospitalized and given the drug at a dose of 700 mg/m² infused in 50 ml of normal saline solution over 30 minutes. After infusion, patients were hydrated with 500–1000 ml of normal saline solution. A preinfusion blood sample was obtained as well as a minimum of eight blood samples drawn in the first 24 hours after infusion. Additional samples were obtained for 144 hours (patients 2 and 3) and for 2 weeks (patients 4–6). Cumulative urine samples were collected by spontaneous voiding, and aliquots of the samples were stored at –5°C. Blood samples (7 ml) were collected and centrifuged (at 1300 × *g* for 4 minutes) in polystyrene tubes containing 0.5 ml of citrate-dextrose-phosphate anticoagulant solution. The resulting plasma solution was frozen until analyzed (within 2 weeks).

Method of Analysis

The analytic procedure used to measure methyl-GAG in both plasma and urine has been described in detail elsewhere (8). Methyl-GAG is separated from the biologic matrix by retention on a small (2 × 0.02-cm) column packed with weak cation exchange resin (Bio-Rex-70, BioRad Laboratories). The analyte is then eluted quantitatively from the column with 0.5-N hydrochloric acid. Separation and quantitation are accomplished by paired-ion reverse-phase high-performance liquid chromatography on a 30-cm RP-18 column (Waters Assoc) with the mobile phase consisting of MeOH:H_2O:HOAC (17.5:82.0:0.5) containing 2.5-mM sodium pentane sulfonate. Spectrophotometric absorbance of the eluent was monitored at 280 nm. Concentration measurements could be made with a precision of ± 4% by comparison of peak areas (determined with a Varian CDS 111-C electronic integrator), with standard curves prepared in plasma and urine.

Data Analysis

Plasma level-time curves, following termination of infusion, were subjected to pharmacokinetic analysis using a nonlinear regression program (NONLIN) (9) on a Honeywell 66/60 digital computer. All data were fit to a biexponential equation of the form $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, where C_p is total plasma concentration, α and β are the apparent distribution and elimination rate constants, respectively, obtained for this model equation, t is time, and A and B are constants of integration. Since all doses were administered as half-hour infusions, the zero-time intercepts were obtained by correcting the computer-generated intercepts according to the method outlined by Gibaldi and Perrier (10). Figure 1 shows a typical fit of the plasma level data in one patient (No. 6).

RESULTS

Table 1 presents the plasma level-time data obtained in six patients following a 0.5-hour infusion of 700 mg/m² of methyl-GAG. The zero time point represents the end of the infusion period. Drug concentration declined rapidly in the first 24 hours but subsequently decreased at a much slower rate. Because of the unexpectedly high drug levels documented at 144 hours in both the plasma and urine of patients 2 and 3, the sampling time was extended to 2 weeks in the next three patients. Since patients were being treated every 2 weeks, samples were not obtained beyond this time point.

The pharmacokinetic parameters obtained from the six patients by the method detailed in the Data Analysis section are presented in table 2. Such calculations yield a range of α-phase half-lives of 20–38 minutes and β-phase half-lives of 20–244 hours. Considering only the patients with plasma samples available through 13 days (patients 4–6), the range of β-phase half-lives is 136–224 hours. It appears that a true terminal phase may not be defined in patients 1–3 since plasma samples were not obtained beyond 144 hours.

The cumulative urinary excretion of intact methyl-GAG (expressed as percent of dose) is reported in

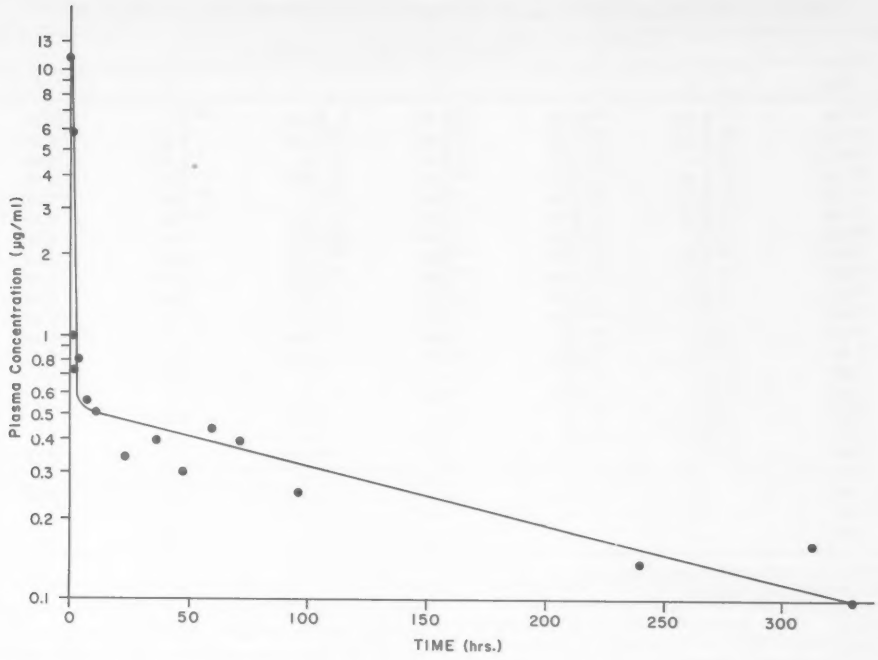

FIGURE 1.—Plasma level-time curve following 0.5-hr infusion of 700 mg/m^2 of methyl-GAG (patient 6). Solid curve = computer-generated equation $C_p = 6.92e^{-1.27t} + 0.53e^{-0.005t}$.

table 3. As with the plasma samples, urine was collected for longer time periods in patients 4–6 than in patients 1–3. Approximately 10% of the administered dose is excreted in the first 18 hours after infusion. Two weeks after administration, only about 40% of the dose can be accounted for through cumulative urine monitoring.

Clinically, all patients experienced periorbital flushing and warmth half way into the infusion, which resolved completely within 15 minutes after the infusion. In another group of patients, this side effect was minimized when the infusion time was increased to 45 minutes. Two patients (Nos. 4 and 5) experienced moderate nausea and vomiting 2–4 days after treatment which persisted for 2 days. There was no hematologic toxicity. One patient (No. 1) experienced a severe peripheral neuropathy of the lower extremities but had no other side effects.

After two doses of the drug (1 month) there were no objective responses. Five patients had increasing disease, and one patient with renal cell carcinoma has had stable disease for 14+ weeks as judged by pulmonary nodules.

DISCUSSION

The plasma level data shown in table 1 and the resultant pharmacokinetic parameters in table 2 illustrate that methyl-GAG plasma levels decline in a multi-exponential fashion with extensive distribution and a very long terminal half-life. From these data, it is not possible to state with certainty at what point a true terminal elimination phase is reached. It is clear, however, that such a phase has not been reached in patients 1–3 over the time course studied. The data from patients 4–6 with resultant β-phase half-lives of 224, 173, and 136 hours, respectively, demonstrate the long elimination half-life of methyl-GAG.

The plasma level-time data further indicate that the plasma is rapidly cleared of significant quantities of the administered dose, although drug is still detectable 13 days after administration. For example, at 2.5 hours after infusion, plasma levels range from only 6% to 20% of their time-zero values. Since there is evidence that methyl-GAG is not significantly bound to plasma proteins (K. C. Marsh,

TABLE 1.—Drug concentration* in plasma following 0.5-hr infusion of 700 mg/m² of methyl-GAG

Time (hrs)	Concentration (μg/ml) in patient No.—					
	1	2	3	4	5	6
0†	9.01‡	16.76	12.75	22.83	—	11.24
0.5	5.35§	6.95	4.46	9.39	4.48	5.95
1.0	—	4.35	4.51	—	1.21¶	0.99
2.5	1.82	2.17	1.87	2.03	1.13	0.74
4.5	1.29	1.49	1.34	—	0.67	0.81
7.5	1.12	0.84	0.74	0.84	0.47	0.56
12.0	—	0.85	0.46	0.53	0.46	0.51
23.5	0.56	0.43	0.50	0.19	0.34	0.34
36	—	0.32	0.26	0.26	0.29	0.39
48	0.27	0.39	0.29	0.30	0.49	0.30
60	—	0.25	0.38	0.10	—	0.44
72	—	0.17	0.26	0.35	—	0.40
96	—	0.33	0.37	0.22	0.30	0.25
120	—	—	—	—	0.40	—
144	—	0.57	0.32	—	—	—
168	—	—	—	0.21	0.28	—
216	—	—	—	—	0.17	—
240	—	—	—	0.09	—	0.14
264	—	—	—	—	0.27	—
312	—	—	—	0.11	—	0.16
337	—	—	—	—	—	0.10
360	—	—	—	—	0.05	—

*Determined as described previously (8).
†End of the 0.5-hr infusion.
‡0.17 hrs.
§0.8 hrs.
¶1.17 hrs.

TABLE 2.—Experimental parameters* from the fit of plasma concentration to the equation $C_p = Ae^{-\alpha t} + Be^{-\beta t}$†

Patient No.	A (μg/ml)	α (hrs^{-1})	B (μg/ml)	β (hrs^{-1})
1	11.61	1.04	1.41	0.04
2	10.79	0.87	0.61	0.01
3	10.47	0.97	0.48	0.007
4	17.07	0.94	0.28	0.003
5	14.62	2.12	0.52	0.004
6	6.92	1.27	0.53	0.005

*Computer fitting as described in the Data Analysis section.
†Correlation coefficient $r^2 \geq 0.9$ was obtained for all patients.

A. J. Repta, and L. Sternson, unpublished data) nor biotransformed in vivo (11), extensive extravascular distribution of methyl-GAG is indicated. These findings in man are consistent with the animal data of Oliverio et al (6).

The urinary excretion data in table 3 suggest that substantial quantities of drug persist in the body 2 weeks after the dose, although plasma levels rapidly decline within the first 24 hours. Patient 5, who exhibited the most extensive excretion of methyl-GAG, had retained almost 60% of the administered dose distributed in the body after 14 days, assuming urinary excretion is the primary route of elimination (6). Such findings demonstrate that plasma level measurements alone may not be an adequate indication of methyl-GAG accumulation that might occur with subsequent doses. Certainly, such findings need to be considered in selecting dose size and frequency in multiple-dose methyl-GAG protocols.

Although the data reported here are preliminary and represent a relatively small patient population, the ability to monitor plasma levels and excretion patterns of methyl-GAG suggests that the drug is rapidly and extensively distributed to extravascular

TABLE 3.—Cumulative urinary excretion (% of dose) following 0.5-hr infusion of methyl-GAG (700 mg/m²)

Time (days)	Cumulative % of dose in patient No.—					
	1	2	3	4	5	6
0.25	3.25	3.46	4.19	4.65	1.78	3.16
0.50	7.0	7.87	7.23	8.87	5.14	6.50
0.75	9.5	11.02	9.14	11.45	8.19	8.04
1.0	10.83	12.51	10.54	12.69	9.34	9.31
1.5	13.95	14.91	12.52	14.29	12.45	11.71
2	16.28	17.19	14.52*	16.20	15.02	12.57
3	—	20.10	17.88	19.62	18.54	14.82
4	—	21.80	20.04	22.40	23.39	16.25
5	—	23.94	23.21	24.98	27.63	18.08
6	—	26.01	26.05	27.27	31.05	19.63
7	—	27.16	27.79	28.67	33.21	20.90
8	—	28.33	28.77	30.54	34.92	21.61
9	—	—	—	32.46	36.46	22.18
10	—	—	—	33.73	37.44	23.51
11	—	—	—	35.11	39.26	24.41
12	—	—	—	36.33	40.71	24.98
13	—	—	—	37.38	42.50	25.37
14	—	—	—	—	43.45	26.10

*Estimated (urine sample not obtained).

sites. In addition, plasma level-time curves indicate the elimination half-life (β) of methyl-GAG is at least 1 week. Finally, a significant fraction of the administered dose persists in the body (as intact drug) for ≥ 2 weeks. Additional data are needed to determine the effects of subsequent doses on the disposition and excretion patterns of methyl-GAG. In the interim, however, investigators should note the very long half-life and high fraction of dose remaining in the body, indicating possible accumulation, when modifying dosing intervals in future methyl-GAG protocols.

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Toxicity and Immunosuppressive Activity of Binary Combinations of 2′-Deoxycoformycin and 2′-Deoxyadenosine[1,2]

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SUMMARY

Treatment of mice with 2′-deoxycoformycin (dCf) for 5 days produced inhibition of spleen and lymph node adenosine deaminase (E. C. 3.5.4.4) activity but no hematologic toxicity or weight loss. A 64-fold elevation of erythrocyte dATP was observed. However, if mice were injected with 2′-deoxyadenosine (AdR) in combination with dCf, weight loss, hematologic toxicity, and liver cell necrosis occurred. These mice had a severe blood coagulation defect and a 73-fold elevation of plasma alanine transaminase activity, plasma prealbumin became undetectable, and erythrocyte dATP levels were elevated 1500-fold. Death during treatment appeared to be from acute liver failure since bone marrow toxicity was only detected following termination of treatment. These effects were not seen in mice receiving adenosine in combination with dCf. dCf, either alone or in combination with AdR, inhibited the contact sensitization to oxazalone in mice. The inhibition was associated with signs of systemic toxicity which were more pronounced in the combination-treated groups. If dATP is the toxic metabolite accumulated in the malignant cells of patients treated with dCf, we propose that AdR supplementation of treatment should be considered with extreme caution since severe damage to normal tissues might result.

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Adenosine deaminase (ADA) (E. C. 3.5.4.4) activity has been shown to be particularly high in T-cell lymphoblasts, and this may prove to be a useful property in the characterization of acute lymphocytic leukemia (1,2). Furthermore, this enzyme also represents a potential target for treatment of T-cell leukemia. The clinical pharmacology and toxicity of the tight-binding ADA inhibitor 2′-deoxycoformycin (dCf) has been studied in patients with a variety of advanced malignant conditions, including leukemia (3). The predominant toxic effects were lymphopenia and some hyperuricemia. dCf has also been used clinically in patients with T-cell leukemia in the terminal stage (4,5), in which some lymphocytotoxicity was also observed.

Many investigators have failed to observe an inhibition of mitogen-mediated lymphocyte blastogenesis using either dCf or coformycin in vitro (6–10). On the other hand, Chassin et al (11) have described an inhibition of response to nonspecific mitogens, although at somewhat higher concentrations of dCf. This may imply that the inhibition is due to effects other than binding to ADA. However, there remains the possibility that ADA inhibition may affect the maturation of precursor lymphocytes as shown by Ballet et al (7).

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Studies on the relationship between purine metabolism and immune function have been stimulated following the observations that an absence of ADA or of purine nucleoside phosphorylase (E. C. 2.4.2.11) activity is linked to inherited immune-deficiency diseases in children (12,13). Immune dysfunction associated with ADA and purine nucleoside phosphorylase deficiency is thought to be mediated by accumulation of dATP and dGTP, respectively (14,15). These deoxynucleotides have been shown to inhibit ribonucleotide reductase (E. C. 1.17.4.1) and subsequent DNA synthesis (16). An alternative hypothesis for T- and B-cell deficiencies arising from an absence of ADA is that 2′-deoxyadenosine (AdR) inactivates *s*-adenosyl homocysteine hydrolase (E. C. 3.3.1.1), leading to inhibition of nucleic acid methylation (17). It is well-documented that the erythrocytes of these children contain increased levels of dATP (14,18).

It is possible that a biochemical consequence of exposure to dCf is the buildup of intracellular dATP leading to inhibition of ribonucleotide reductase. Studies from these laboratories indicate that dCf treatment leads to a profound elevation of erythrocyte dATP in mice and in man (6). dCf potentiates the cytotoxicity of AdR against L1210 cells in culture, in association with a marked elevation of intracellular dATP (19). In mice bearing L1210 tumors, treatment with binary combinations of dCf and AdR leads to a significant extension of survival; whereas administration of either agent alone is without effect (19).

These observations imply that combinations of AdR and dCf may have clinical utility. However, we wish to report here that under certain conditions such treatments can prove to be profoundly toxic. In addition, we report preliminary observations on the immunosuppressive activities of dCf and dCf and AdR combinations.

MATERIALS AND METHODS

dCf was supplied by the Drug Evaluation Branch, Developmental Therapeutic Program, National Cancer Institute, Bethesda, MD. Adenosine (AR) and AdR were purchased from the Sigma Chemical Co, Ltd, London, England.

Toxicity Studies

BDF_1 mice, 10–12 weeks of age, received dCf at a dose of 0.27 mg/kg daily for 5 days by ip injection. In addition, 30 minutes and 6 hours after each dCf injection, mice received isotonic solutions of AR at doses of 267 or 26.7 mg/kg and AdR at doses of 267 or 26.7 mg/kg. Injection volumes were 0.01 ml/g of body weight; control groups received saline only. Ten mice from each group were set aside and weighed daily for 12 days, and then every other day for an additional 11 days. Groups of ten mice were killed on Day 5 following their first nucleoside or deoxynucleoside injection of that day. The remaining ten mice were killed 2 days after discontinuation of treatment. The exception to this was the group of 20 mice receiving AdR at a dose of 267 mg/kg: during treatment they rapidly became debilitated and lost weight. Surviving animals were killed on the fourth day of treatment, following their first AdR injection of that day. The survivors of an additional group of ten mice receiving 267 mg/kg of AdR were killed 1 day after discontinuation of treatment. Animals were bled for assessment of erythrocyte dATP levels, hematology, and liver function tests. Femoral bone marrow smears were also examined. Heart plus thymus, spleen, liver, kidneys, and lymph nodes were removed, were fixed in Bouin's fluid, and were processed, embedded, and sectioned by routine methods prior to subsequent histologic examination. Some spleens and lymph nodes were retained for measurement of ADA activity.

Contact Sensitization

According to the method of Asherson and Zembala (20), groups of ten male BALB/c mice, weighing 18–22 g, were sensitized to either 3% or 1% oxazalone (British Drug Houses) in an ethanol/cellulose/Tween 80 vehicle by painting the shaved flank skin. Six days after sensitization, mice were challenged by painting one ear with either 3% or 1% oxazalone in the same vehicle. The other ear was painted with the vehicle alone. The difference in thickness between the control and challenged ears was measured 24 hours later. Nonspecific irritation was assessed in control groups of five mice by ear painting without prior sensitization.

Drug Treatment

dCf was given ip at a dose of 1 mg/kg. A second group of mice received the dCf followed at 1 and at 6 hours by a dose of 26.7 mg/kg of AdR, also given ip. Daily treatment was started 4 days before sensitization and was continued until challenge 6 days after sensitization. In mice given both dCf and AdR there was evidence of toxic effects, in both weight loss and poor condition of the mice. The schedule was therefore reduced to a single injection of AdR given 1 hour after the dCf from 1 day before sensitization until challenge 6 days after sensitization. Three of the ten mice in each group receiving the

combination of drugs died during this period. Postmortem examination was conducted on all mice, and the only consistent finding was a great reduction in the size of the thymus in groups receiving dCf either alone or in combination with AdR.

ADA Estimation

Spleen and lymph node ADA activity was assayed as described by Smyth and Harrap (21). Spleen ADA was extracted by the method of Jackson et al (22). Axillary and inguinal lymph nodes were homogenized in 0.15 M sodium phosphate buffer, pH 7.1 (20% wt/vol), using a Potter-Elvehjem homogenizer (ten strokes). The homogenate was sonicated at 20 KHz for 60 seconds in ice and was centrifuged at 600 × *g* for 10 minutes at 4°C. The resultant supernatant was used for assay.

dATP Assay

Erythrocyte dATP was extracted as previously described (6) and was assayed according to the method of Tattersall and Harrap (23).

Plasma Protein Electrophoresis

Cellulose acetate electrophoresis of plasma proteins was carried out as described by Harris and Kohn (24). The results obtained with human serum indicated that the assessment of pre-albumin levels by visual inspection correlated well with those obtained by immunologic methods.

Alanine Transaminase and Blood Coagulation Studies

Plasma alanine transaminase levels were estimated by the method of Wallnöffer et al (25). Prothrombin time, kaolin partial thromboplastin time, factor V, factor X, and fibrinogen titer were assayed as described by Dacie and Lewis (26).

RESULTS

Figure 1 shows that both during and after treatment there was < 10% weight loss in all groups, with the exception of mice injected with AdR at a dose of 267 mg/kg in combination with dCf. Animals

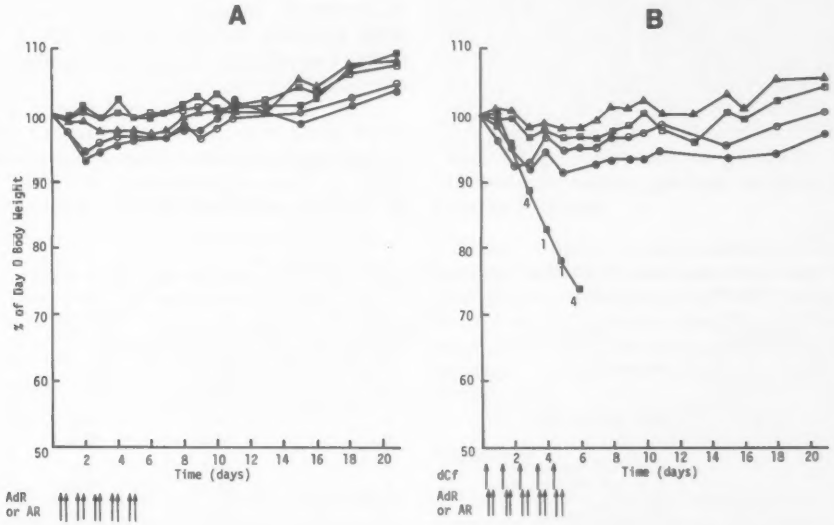

FIGURE 1.—Body weight curves of BDF_1 mice receiving nucleosides alone (A), or nucleoside and dCF combinations (B), for 5 days. A: △——△ = saline alone (10 of 10 survivors); ○——○ = AR, 26.7 mg/kg twice daily (10 of 10 survivors); ●——● = AR, 267 mg/kg twice daily (10 of 10 survivors); □——□ = AdR, 26.7 mg/kg twice daily (10 of 10 survivors); ■——■ = AdR, 267 mg/kg twice daily (10 of 10 survivors); B: △——△ = saline twice daily + dCf, 0.27 mg/kg once daily (10 of 10 survivors); ○——○ = AR, 26.7 mg/kg twice daily + dCf, 0.27 mg/kg once daily (10 of 10 survivors); ●——● = AR, 267 mg/kg twice daily + dCf, 0.27 mg/kg once daily (10 of 10 survivors); □——□ = AdR, 26.7 mg/kg twice daily + dCf, 0.27 mg/kg once daily (10 of 10 survivors); ■——■ = AdR, 267 mg/kg twice daily + dCf, 0.27 mg/kg once daily (0 of 10 survivors). Nos. = No. of deaths on days indicated. Arrows = injection times.

TABLE 1.—Spleen ADA activity following treatment with dCf alone or combined with AR or AdR*

Group No.	Treatment	Day after 1st injection	Specific activity†	% control activity
1	Saline	4	163.6	100
		6	143.0	100
2	dCf, 0.27 mg/kg	4	3.2	1.9
		6	29.6	20.7
3	dCf, 0.27 mg/kg	4	5.6	3.5
	+ AR, 26.7 mg/kg	6	33.6	23.5
4	dCf, 0.27 mg/kg	4	3.0	1.8
	+ AR, 267 mg/kg	6	38.9	27.2
5	dCf, 0.27 mg/kg	4	5.0	3.1
	+ AdR, 26.7 mg/kg	6	39.9	27.9
6	dCf, 0.27 mg/kg	3	1.8	1.1
	+ AdR, 267 mg/kg	5	31.3	21.9

*Results = mean values of replicate assays from 1 experiment.
†mmols of inosine formed/hr/g of wet tissue.

in this group lost weight progressively as treatment continued; by Day 6 the weights were 74% of the pretreatment values. There were no survivors in this group. A short-lived toxic effect was noted in mice receiving AR at a dose of 267 mg/kg alone or in combination with dCf: the animals became immobile for 30 minutes after each nucleoside injection but then recovered. The physiologic role of AR has been well-documented, and the toxic effect we observed might be accounted for by a change in the rate of blood flow in heart and brain and/or altered neurotransmission in the brain (27–30). Except for the toxic effects observed in the mice receiving AR at a dose of 267 mg/kg, no other toxic effects were observed in animals receiving nucleoside alone at any dose.

Spleen and lymph node ADA activity was markedly inhibited during treatment in all groups, being reduced to < 5% of control activity (tables 1 and 2). Forty-eight hours after discontinuation of treatment to groups 2–5, spleen ADA activity had recovered to 20.7%–27.9% of control activity and lymph node ADA activity had recovered to 16.5%–22.4%. In group 6, spleen and lymph node ADA activities were 21.9% and 6.7% of control activity, respectively, 24 hours after discontinuation of treatment.

After treatment with dCf alone, erythrocyte dATP levels increased from 0.1 to 20.1 nmols/ml of packed erythrocytes on Day 4 (fig 2). When dCf treatment was supplemented with AdR (26.7 and 267 mg/kg), additional marked elevations of eryth-

TABLE 2.—Lymph node ADA activity following treatment with dCf or combined with AR or AdR*

Group No.	Treatment	Days after 1st injection	Specific activity†	% control activity
1	Saline	4	165.6	100
		6	171.9	100
2	dCf, 0.27 mg/kg	4	5.9	3.6
		6	38.5	22.4
3	dCf, 0.27 mg/kg	4	3.7	2.2
	+ AR, 26.7 mg/kg	6	31.1	18.1
4	dCf, 0.27 mg/kg	4	3.5	2.1
	+ AR, 267 mg/kg	6	30.4	17.7
5	dCf, 0.27 mg/kg	4	7.4	4.5
	+ AdR 26.7 mg/kg	6	28.4	16.5
6	dCf, 0.27 mg/kg	3	4.5	2.7
	+ AdR, 267 mg/kg	5	11.5	6.7

*Results = mean values of replicate assays from 2 separate experiments.
†mmols of inosine formed/hr/g of wet tissue.

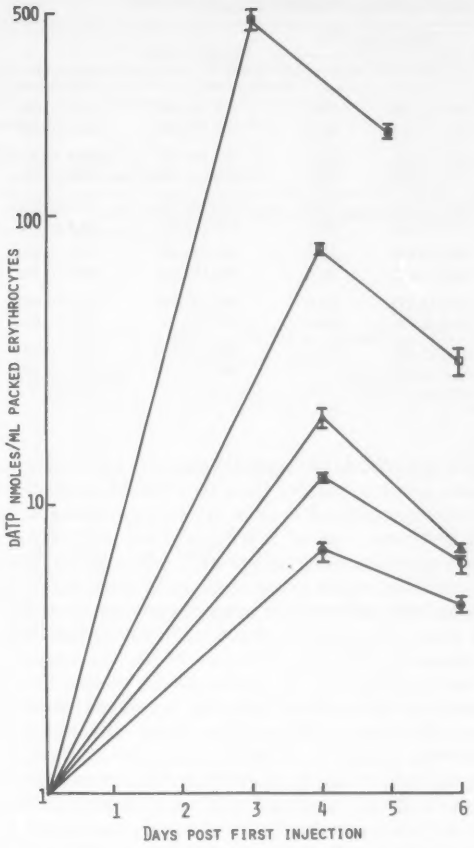

FIGURE 2.—Erythrocyte dATP levels in mice receiving dCf alone or combined with AR or AdR for 5 days. Schedule as follows: ▲——▲ = dCf, 0.27 mg/kg once daily; ○——○ = dCf, 0.27 mg/kg once daily + AR, 26.7 mg/kg twice daily; ●——● = dCf, 0.27 mg/kg once daily + AR, 267 mg/kg twice daily; □——□ = dCf, 0.27 mg/kg once daily + AdR, 26.7 mg/kg twice daily; ■——■ = dCf, 0.27 mg/kg once daily + AdR, 267 mg/kg twice daily. Results = mean values of triplicate determinations from 2 separate experiments, 5 mice being used at each point. Bars = SEM.

rocyte dATP were observed (to 77.1 and 478.3 nmols/ml, respectively). Interestingly, erythrocyte dATP levels in mice treated with AR plus dCf were lower than the levels achieved in mice treated with dCf alone. This may be due to competition of administered AR with the phosphorylation of endogenous AdR.

No leukopenia or other hematologic toxic effects were observed in mice treated with dCf at a dose of 0.27 mg/kg (table 3). Although the wbc count fell slightly in mice treated with 26.7 and 267 mg/kg of AR and 26.7 mg/kg of AdR, the lymphocyte:neutrophil ratio remained unchanged. The main hematologic effect was noted in mice receiving 267 mg/kg of AdR plus dCf. Leukopenia was very pronounced in two mice, in which the wbc count decreased to 0.7×10^9/liter and was associated with agranulocytosis 24 hours after termination of treatment. In the remaining animals in this group the wbc counts decreased, but to a less marked degree, and neutrophils were still present in reasonable number, although a technical neutropenia was produced. The femoral marrows of these mice, however, and, in particular, in the two mice with marked neutropenia, showed cytotoxic effects with vacuolation and some giant metamyelocytes, consistent with the findings in the peripheral blood.

Another hematologic disturbance, confined to the group of animals receiving 267 mg/kg of AdR in combination with dCf, was the failure of whole blood to clot over a 3-hour observation period; the clotting time of all other treatment groups was normal. The small reduction in platelet counts is not sufficient to account for this observation; it is more consistent with the loss of clotting factors, attributable to liver cell damage. Further examination of this effect is shown in table 4. It is apparent that a severe coagulation defect was produced in the treated mice and that it was accompanied by a 73-fold elevation in plasma alanine transaminase activity. These results are consistent with an acute hepatic dysfunction and cell necrosis.

Plasma Proteins

Figure 3 shows a disappearance of the plasma prealbumin band in two mice receiving 267 mg/kg of AdR in combination with dCf, compared to a control mouse (under the conditions of the technique used). In terms of human pathology, a significant decrease in prealbumin is indicative of severe liver damage (24).

Histology

The dominant changes were seen in the combination-treated group (No. 6), and they involved the liver, kidneys, and lymphoreticular tissues. Treatment with either dCf, AR, or AdR alone caused little or no morphologic hepatic change except a mild periportal infiltration with small normal cells and an occasional polymorphonuclear leukocyte. Treatment with AdR (267 mg/kg) and dCf (0.27 mg/kg) in combination, however, resulted in hepatic cell

TABLE 3.—Hematologic effects following treatment with dCf alone or combined with AR or AdR*

Treatment	No. of mice/group	Day after 1st injection	Total wbc count (cells × 10^9/liter)	Differential (%)		Platelet count (cells × 10^9/liter)	Hemoglobin (%)
				Lymphocytes	Neutrophils		
Saline	5	4	9.7 (0.85)†	76.2 (2.13)	23.8	722 (15.48)	15.8 (0.22)
	5	6	8.1 (0.65)	74.4 (1.36)	25.6	742 (31.80)	15.6 (0.20)
dCf, 0.27 mg/kg	5	4	11.3 (1.40)	76.2 (1.35)	23.8	722 (20.65)	15.1 (0.24)
	5	6	10.0 (0.87)	77.2 (1.28)	22.8	654 (27.90)	14.9 (0.23)
dCf, 0.27 mg/kg	5	4	6.4 (0.60)	74.4 (2.04)	25.6	738 (28.79)	15.1 (0.26)
+ AR, 26.7 mg/kg	5	6	7.8 (0.46)	74.0 (1.51)	26.0	518 (66.0)	15.4 (0.19)
dCf, 0.27 mg/kg	5	4	5.3 (0.63)	75.2 (0.86)	24.8	685 (35.90)	14.8 (0.40)
+ AR 267 mg/kg	5	6	6.5 (0.80)	74.0 (1.95)	26.0	762 (41.20)	16.0 (0.15)
dCf, 0.27 mg/kg	5	4	5.9 (0.95)	75.0 (1.97)	25.0	650 (96.90)	15.2 (0.43)
+ AdR 26.7 mg/kg	5	6	8.1 (1.24)	75.2 (2.08)	24.8	748 (34.0)	14.0 (1.0)
dCf, 0.27 mg/kg	6	3	2.48 (0.55)	49.0 (6.52)	51	499 (48.70)	16.2 (0.72)
+ AdR, 267 mg/kg	10	5	1.54 (0.27)	99.2 (0.42)	0.8	397 (33.40)	13.1 (0.34)

*Values = group arithmetic mean of observations on individual mice.
†Values in parentheses = SEM.

damage. The least observed change consisted of swelling of the parenchymal liver cells together with fatty change. This change started in the periportal area of each lobule, with increasing severity extending towards the central vein, and could, in some cases, come to involve the entire lobule. Some liver cells also exhibited hyaline cytoplasmic changes. In all instances, foci of individual liver cell necrosis were noted: in many, apparently following the fatty change, extensive areas of necrosis were noted, to which little inflammatory responses were observed. In most instances the kidneys had a normal morphologic appearance. In some of the combination-treated group (No. 6), although the glomeruli and proximal convoluted tubules appeared normal, marked cloudy swelling of the distal convoluted tubule was apparent. In almost all animals, the lymphoreticular tissues appeared normal. However, in some animals receiving dCf or dCf plus AdR a marked decrease in cellularity was noted which affected the lymphocyte population in particular.

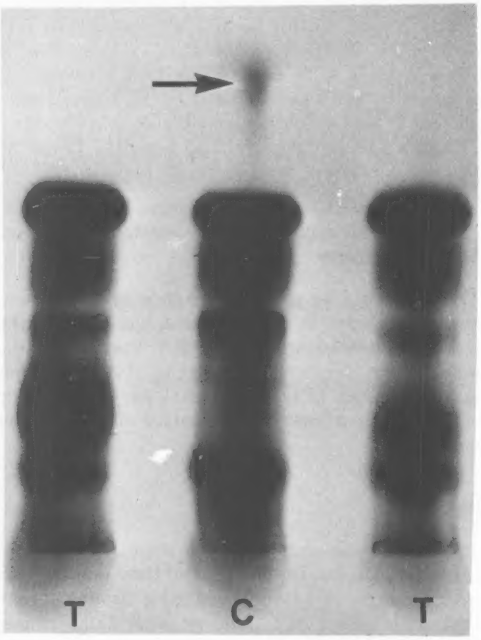

FIGURE 3.—Mouse plasma protein separation pattern obtained using CAM electrophoresis (ponceau S strain). Arrow = prealbumin fraction, clearly seen in control mouse (C). Patterns on either side are from mice receiving dCf + AdR at 267 mg/kg (T). The prealbumin fraction is absent in these cases.

Contact Sensitization

The contact responses of mice receiving both dCf and AdR were more variable than those of other groups (table 5). Despite this, the reduction in contact sensitization seen in mice receiving either dCf alone or combined with AdR was highly significant ($P > 0.001$) when 3% oxazalone was employed. The reduction was less pronounced when 1% oxazalone was used.

DISCUSSION

Following a phase I clinical study of dCf, Smyth et al (3) demonstrated lymphocytotoxicity in patients receiving 0.25 mg/kg for 5 days. The erythrocyte dATP levels in these patients were profoundly increased, to a maximum of 975 nmols/ml

TABLE 4.—Blood coagulation studies and plasma alanine transaminase levels*

Treatment	Prothrombin time (secs)	Kaolin partial thromboplastin time (secs)	Factor V (%)	Factor X (%)	Fibrinogen titer	Alanine transaminase (IU/liter)
Saline	11	33	80	110	128	13
dCf, 0.27 mg/kg† + AdR, 267 mg/kg	> 180	> 300	10	10	1	946

*Values = mean of duplicate assays on pooled plasmas from 8 animals.
†4 days after 1st injection.

TABLE 5.—Effects of dCf alone or combined with AdR on oxazalone-induced skin sensitization in the mouse*

Treatment with oxazalone	Drugs	Difference in ear thickness (μ) measurement + SE	*P* value	Mean body wt (g) ± SE	*P* value
1% (× 2)	—	135 ± 13		21.8 ± 0.4	
1% (× 2)	dCf	83 ± 16	> 0.05	15.0 ± 0.8	> 0.001
1% (× 2)	dCf + AdR	118 ± 24	NS†	13.4 ± 1.0	> 0.001
1% (× 1)	—	12 ± 1	> 0.001	25.0 ± 0.8	> 0.01
3% (× 2)	—	351 ± 17		22.1 ± 0.5	
3% (× 2)	dCf	179 ± 18	> 0.001	15.5 ± 0.7	> 0.001
3% (× 2)	dCf + AdR	111 ± 22	> 0.001	13.5 ± 1.0	> 0.001
3% (× 1)	—	196 ± 34	> 0.001	21.8 ± 0.5	NS

*Student's t test comparison with appropriate sensitized control.
†NS = not significant.

of packed erythrocytes in one patient during treatment (6). However, as reported here, in mice receiving 0.27 mg/kg of dCf daily × 5, no lymphocytotoxicity was observed, although spleen and lymph node ADA activities were markedly inhibited during treatment, with a concomitant elevation of dATP (but only to 20.3 nmols/ml of packed erythrocytes). In the present investigation we have administered AdR at a dose of 267 mg/kg in combination with dCf and have observed hematologic toxic effects and life-threatening liver damage. Under these circumstances, erythrocyte dATP levels reached 478.3 nmols/ml of packed erythrocytes in the mice, which is comparable with those achieved in patients receiving dCf alone (6). While we have previously shown that the antitumor properties of dCf can be enhanced in the mouse by the concomitant administration of AdR (19), it is clear that the intracellular dATP levels produced in man following the administration of dCf alone are higher than those produced in the mouse. AdR supplementation of dCf treatment in man may therefore result in the accumulation of toxic levels of intracellular dATP which, at present, cannot be predicted accurately from mouse studies.

In view of the parallel experiments reported here, in which mice received AR at a dose of 267 mg/kg in combination with dCf and in which we saw no hematologic or liver toxicity and a smaller increase of erythrocyte dATP levels than with dCf treatment alone, we can only assume that AdR or its nucleotides are responsible for the observed tissue damage. If dATP is the toxic metabolite accumulated, our results suggest that dCf is not selectively lymphocytotoxic and that high dATP levels may result in severe liver damage. Administration of AdR plus dCf, at an appropriate dosage, can lead to liver cell necrosis, confirmed by the histopathology studies, increase in plasma alanine transaminase activity, deletion of plasma prealbumin, and the induction of a profound coagulation defect. The molecular basis for liver toxicity is currently under investigation.

dCf has been reported to be immunosuppressive in a tumor allograft system (31). Our results in a contact sensitization system broadly confirm the latter, but there are difficulties in interpreting the contact sensitization results as a drug-induced immune suppression. Dramatic loss in body weight and deterioration in general condition were associated with drug treatment, particularly with the combination of dCf and AdR. It is therefore possible that inhibition of contact sensitization was secondary to other manifestations of toxicity, rather than to a direct effect of treatment with dCf alone or

combined with AdR on lymphoid tissues. The inhibition of sensitization was less pronounced when 1% oxazalone was used, and only at this concentration was the response free of a considerable nonspecific irritation. The inhibition seen using 3% oxazalone may have included suppression of some nonspecific component of the response.

These observations indicate that any proposed supplementation of clinical schedules containing dCf with nucleosides should be considered extremely carefully. Furthermore, even when using dCf alone it would also be wise to monitor the circulating AdR levels in order to establish "safe" levels of this metabolite during treatment.

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Studies in Mice Treated With ICRF-159 Combined With Daunorubicin or Doxorubicin[1]

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SUMMARY

We have investigated the effect of ICRF-159 on the toxicity of daunorubicin (DR) and doxorubicin (DX) given iv, and the effectiveness of ICRF-159 combined with DR or DX on the growth of transplantable MLV-M (murine leukemia virus-Moloney) leukemia, MS-2 solid sarcoma, and pulmonary MS-2 metastases in mice. The injection of ICRF-159 concurrently with the administration of DR resulted in a marked decrease in the toxicity of the antibiotic. However, when DX was injected concurrently with ICRF-159 an increase in antibiotic toxicity was observed, except when ICRF-159 was employed at a very low dosage. ICRF-159 administered alone did not influence the tumor growth in the systems tested and did not result in antimetastatic activity. In mice bearing transplanted MLV-M leukemia, the effects of the combination of ICRF-159 with DR or DX were not superior to those of DR or DX treatment on either tumor growth or lifespan. The treatment of MS-2 tumor with the ICRF-159 and DX combination neither produced a therapeutic synergism (therapeutic response superior to the maximum response obtainable by either agent independently) nor antagonized the antineoplastic action of DX. A marked inhibition of tumor growth and increase in lifespan were observed in the mice treated with a high dose of DR (10 mg/kg/injection) plus ICRF-159 (50 mg/kg/injection). We have also examined, on MS-2 lung metastases, the effectiveness of surgical-adjuvant combination chemotherapy with DR or DX plus ICRF-159 injected at different times with respect to surgery. A synergistic effect of DX or DR with ICRF-159 was observed when the drug treatment was performed before the surgery, or both before and after the surgery. No synergistic effect of DX or DR with ICRF-159 on MS-2 lung metastases was found when the MS-2 lung metastases were treated after the surgery. A higher antimetastatic activity was observed in the groups treated with a combination of toxic doses of DR and ICRF-159 than in the groups treated with tolerated doses of the antibiotic.

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The anthracycline antibiotics daunorubicin (DR) and doxorubicin (DX) have been used against a large number of human tumors (1,2). Their spectrum of antitumor activity extends from hematologic malignancies to breast and thyroid carcinomas and to bone and soft tissue sarcomas. DX is probably the single agent with the broadest spectrum in cancer chemotherapy, whereas DR is currently used primarily in acute leukemias (1–3). Unfortu-

nately, the therapeutic use of DX and DR has been limited by toxic effects such as myelosuppression and cumulative dose-related cardiotoxicity (2–4). Efforts to reduce the toxicity (especially cardiac toxicity) of these two agents by the synthesis of new anthracycline analogs are currently under way (5). Experiments have also been carried out to study the antidotal activity exerted by the administration of some chemicals like coenzyme Q (6), vitamin E (7), ICRF-159 (8,9), and its *d*-isomer ICRF-187 (10).

Venditti (11) reported that ICRF-159 (Razoxane) combined with a nonlethal dose of DR had greater antileukemic efficacy than that achieved with DR alone, and when ICRF-159 was combined with a lethal dose of DR, the former protected against the toxicity of the latter. Woodman et al (12) found that nonleukemic mice could tolerate several times more DR when ICRF-159 was administered concurrently (both drugs injected ip). ICRF-159 was also described to enhance the effectiveness of DX against L1210 leukemia, even if no reduction of DX toxicity was observed (13,14).

In addition, the combination of ICRF-159 with DX and DR has been shown to reduce the cardiotoxicity of these drugs on the isolated dog heart (8,9). The protective effect of ICRF-159 is of great interest, as this compound has been reported to enhance the antitumor activity of DR and also to exert antitumor (15–17) and antimetastatic (17,18) effects. Clinical activity of the combination therapy with DX and ICRF-159 is also under study (19,20).

The above findings have led us to investigate the effect of ICRF-159 on the toxicity, antitumor activity, and antimetastatic effectiveness of DR and DX given iv to normal mice and to mice bearing transplantable tumors.

MATERIALS AND METHODS

Animals.—The animals used throughout these experiments were 6–8-week-old syngeneic BALB/c mice, obtained from Charles River Breeding Laboratories, Calco, Italy.

Tumors.—The following tumors were used: (*a*) Transplantable MLV-M (murine leukemia virus-Moloney) leukemia obtained by serial passage of sterile homogenates of spleen and lymph nodes from BALB/c mice in which leukemia developed following infection with MLV-M; leukemic cells were injected iv in adult BALB/c mice (10^3 cells/mouse); and (*b*) MS-2 tumor produced in BALB/c mice by transplant of in vitro cultured cells (MS-2 cells) derived from a primary MSV-M tumor in mice (21,22), and maintained by serial im passages of a tumor homogenate into the right hind leg; the characteristics of MS-2 tumor were previously described (23,24). Tumor growth and median survival times were calculated as described by Geran et al (25). For antimetastatic activity experiments, the MS-2 tumor cell suspension was injected into the footpad (10^6 cells/mouse).

Surgical tumor removal.—The tumor was removed surgically as previously described (24). Animals were anesthetized with sodium thiopental (Farmotal; Farmitalia, Milan, Italy). The tumor-bearing foot and leg were washed with 95% alcohol. Sterile, natural surgical thread was used to tie the femoral artery and adjacent blood vessels. The muscle and femur were cut out with sterile scissors and the wound was closed with metal wound clips. The excision line was made all around the upper thigh as far as possible above the area of tumor growth (the popliteal lymph nodes were included in the line of amputation). The wound was treated before and after the application of metal clips with an antibacterial powder. Surgical removal resulted in < 10% mortality (any mortality was probably due to anesthesia); no primary tumor recurrence or bacterial infections were observed.

Assay for metastatis detection.—Mice were killed by cervical dislocation. The lungs were removed aseptically, minced, inserted into a syringe with a small amount (0.5–0.6 ml) of 199 medium, and injected sc into syngeneic normal BALB/c mice. The lungs of each animal were bioassayed individually. Mice were observed for 100–120 days after tissue implantation. The tumor growth after sc implant of lungs was the criterion for the presence of lung metastatic foci.

Drugs.—DR and DX hydrochloride (Farmitalia Carlo Erba, Milan, Italy) were dissolved in distilled water or physiologic solution and were injected iv in a volume of 10 ml/kg of body weight. ICRF-159, generously supplied by Dr. K. Hellmann, Imperial Cancer Research Fund, London, England, was suspended in sterile 0.3% (wt/vol) carboxymethyl cellulose in saline, and was injected ip. The ICRF-159 was administered a few minutes after injection of DR or DX.

Statistical analysis.—Statistical significance was evaluated by Student's t test or by Mussett's test (26).

RESULTS

Toxicity of DR and DX Plus ICRF-159 in Normal Mice

Table 1 shows toxicity data for DR, DX, and ICRF-159 administered at repeated doses in non-

TABLE 1.—Effect of treatment with ICRF-159, DR, and DX administered alone or in combination to normal BALB/c mice

ICRF-159		Toxic deaths* from DR† at various doses (mg/kg/injection)			Toxic deaths* from DX† at various doses (mg/kg/injection)			
Dose (mg/kg/injection)‡	Toxic deaths*	8	10	12	5	6	7	8
—	0/10	14/25 (56)	22/25 (88)	25/25 (100)	1/5 (20)	3/25 (12)	6/25 (24)	6/20 (30)
10	0/10	0/10	0/10	2/10 (20)	ND§	0/10	0/10	2/10 (20)
30	0/10	0/10	0/10	2/10 (20)	ND	3/10 (30)	9/10 (90)	8/10 (80)
50	0/10	0/10	0/10	2/10 (20)	ND	3/10 (30)	4/10 (40)	7/10 (70)
75	0/10	0/10	0/10	0/10	ND	5/10 (50)	9/10 (90)	7/10 (70)
150	0/15	1/15 (6.6)	3/15 (20)	11/15 (73)	3/5 (60)	11/15 (73)	15/15 (100)	10/10 (100)

*Values = No. of toxic deaths/total No. of mice treated. (% mortality). Mice were observed for 100–120 days after the last treatment.

†Treatment iv on Days 1, 4, and 7.

‡Treatment ip on Days 1, 4, and 7.

§ND = not done.

tumor-bearing BALB/c mice. The mice were treated on Days 1, 4, and 7. ICRF-159, administered at dose levels ranging from 10 to 150 mg/kg/injection appeared well-tolerated and virtually nontoxic.

The treatment with DR at doses of 8, 10, or 12 mg/kg/injection and with DX at doses of 6, 7, or 8 mg/kg/injection showed a dose-dependent toxicity.

DX at a dose of 5 mg/kg/injection was slightly more toxic than at a dose of 6 mg/kg/injection; this discrepancy was probably due to the small number of mice used in the experiment.

ICRF-159 administered ip at doses ranging from 10 to 150 mg/kg/injection markedly reduced the mortality due to the iv treatment with DR. Antagonism was evident across the ICRF-159 dose range of 10–75 mg/kg/injection; at the higher dose of 150 mg/kg/injection, the effectiveness of ICRF-159 was decreased. In contrast, when ICRF-159 was administered in combination with DX, an increase of the antibiotic toxicity was observed at all doses of ICRF-159 tested, with the exception of the 10-mg/kg/injection dose which showed a slight protective effect.

Activity of DR or DX in Combination With ICRF-159 Against MLV-M Leukemia

In BALB/c mice bearing transplanted MLV-M leukemia (table 2), ICRF-159 did not significantly increase the therapeutic effect of DR, even when ICRF-159 was administered three times at 70 mg/kg/injection. Some degree of synergism between the two agents was observed in the group treated with ICRF-159 and DR at Day 1 after the tumor cell inoculum.

In the groups treated with DX and ICRF-159 at various doses, no increase in the activity of DX was observed but there was an increase of DX toxicity.

Activity of DR or DX in Combination With ICRF-159 Against MS-2 Sarcoma

BALB/c mice bearing MS-2 sarcoma received five repeated treatments at 3–4-day intervals with DX at the tolerated dose of 4 mg/kg/injection and with ICRF-159 at various doses. The data reported in table 3 show that, when administered according to these schedules, DX treatment resulted in a significant reduction in the tumor growth and a statistically significant increase in lifespan, which was, however, not observed in all of the experiments. The combination of DX plus ICRF-159 did not produce any increase in the antitumor activity of DX. With the higher dose of ICRF-159, an increase of toxicity which caused a reduction of the increase in lifespan, was observed.

In a similar experiment (table 4), the antitumor effect of DR alone and combined with ICRF-159 was tested against MS-2 sarcoma. When tolerated doses of DR were employed (5 and 6 mg/kg/injection), no synergism was observed with ICRF-159 (30 and 50 mg/kg/injection). When toxic doses of DR were employed (8 and 10 mg/kg/injection), the combination with ICRF-159 led to decreased toxicity, while the antitumor activity was not abolished. In the group of mice treated with 10 mg/kg/injection of DR plus 50 mg/kg/injection of ICRF-159, a marked inhibition of tumor growth and a marked increase in lifespan were observed. The increase in lifespan (74%) was 2.4-fold higher than the maximum increase in lifespan achieved by DX alone, and twofold greater than the maximum produced by the optimal combination of DX plus ICRF-159 (table 3).

TABLE 2.—Effect of combination treatment with ICRF-159 and DR or DX against MLV-M leukemia

Treatment (mg/kg/injection)						
ICRF-159 ip	DR iv	DX iv	Treatment Day‡	T/C%*	No. of dead mice/ total No. of mice treated†	No. of toxic deaths/ No. of mice treated
100	—		1	100	10/10	
—	12			(152)	5/10	
100	12			(465)	1/10§	
100	—		3	105	10/10	
—	12			(179)	4/10¶	1/10
100	12			(150)	4/10¶	1/10
100	—		10	102	10/10	
—	12			194	10/10	2/10
100	12			161	10/10	
70	—		1, 3, 6	105	10/10	
—	5			(136)	3/10§	
70	5			(297)	4/10¶	
250		—	1	107	10/10	
—		10		(113)	4/10¶	1/10
250		10		(23)	4/10¶	4/10
150		—	1, 3, 6	119	10/10	
—		4		125	10/10	
150		4		50	10/10	10/10

*T/C% = median survival time of treated mice/median survival time of untreated controls × 100. Values in parentheses = data on dead mice only.

†At 108 days after tumor cell injection.

‡No. of days after iv injection of leukemic cells in BALB/c mice.

§$P < 0.01$ (Mussett's test between treated vs control mice).

¶$P < 0.05$.

TABLE 3.—Activity of DX in combination with ICRF-159 against MS-2 sarcoma*

ICRF-159 ip (mg/kg/injection)	DX (mg/kg/injection)	% inhibiton of tumor growth†	%ILS‡
30		3 (21)	3
50		1 (21)	13
150		−13 (40), −13 (31)	−5, 3
—	4	71 (21),§ 70 (40),§ 47 (31)	31,§ 37,§ −2
30	4	64 (21)§	35§
50	4	74 (21)§	34§
150	4	40 (40),§ 40 (31)§	−4, 10

*Treatment every 3 or 4 days, 5 times. Data from 3 experiments. Tumors were measured weekly, starting when the tumor volume became measurable.

†Measurement performed on Day 21, 31, or 40 after im tumor implant. Values in parentheses = Day of measurement.

‡Mean % increase in lifespan.

§$P < 0.01$ (Student's t test vs controls).

Combination Treatment With DR or DX Plus ICRF-159 Against MS-2 Lung Metastases

BALB/c mice were injected in the footpad with 10^6 MS-2 tumor cells. At Day 12, the leg was surgically removed and the mice were treated with the compounds four or five times, starting on Day 1 after tumor removal. Seven days after the end of treatment, the mice were killed and lung metastases were evaluated by bioassay.

The results of two experiments are shown in table 5. ICRF-159 and DR alone did not show any activity. DX alone slightly reduced the incidence of lung metastases. When administered in combination with ICRF-159, a slight increase (which was not statistically significant) was observed in the activity of DR and DX against MS-2 lung metastases.

Table 6 shows the results of DX and ICRF-159 combination chemotherapy applied according to a different schedule of treatment; ICRF-159 was

TABLE 4.—Activity of DR in combination with ICRF-159 against MS-2 sarcoma*

ICRF-159 (mg/kg/injection)	DR (mg/kg/injection)	% inhibition of tumor growth†	%ILS‡
30	—	28 (21), 24 (33)	10.5, 3.5
50	—	1 (21), −0.6 (25)	13, 20
—	5	55 (33)§	20¶
30	5	24 (33)	16
—	6	63 (21)§	30§
30	6	54 (21)§	14¶
50	6	59 (21)§	25§
—	8	80 (25)§	−6
50	8	54 (25)§	34§
—	10	ND	−106
50	10	75 (25)§	74§

*Treatment on Days 1, 4, 7, 10, and 13 after tumor transplant. Data from 3 experiments. Tumors were measured weekly starting when the tumor volume became measurable.
†Values in parentheses = Day of measurement.
‡Mean % increase in lifespan.
§$P < 0.01$ (Student's t test vs controls).
¶$P < 0.05$.

TABLE 5.—Activity of DR or DX in combination with ICRF-159 against MS-2 lung metastases*

ICRF-159 (mg/kg/injection)	DR (mg/kg/injection)	DX (mg/kg/injection)	Lung metastases evaluation† No. of animals with tumor/ No. of mice inoculated‡	%
—	—	—	14/15, 12/12	96.2
150	—	—	7/7, 13/13	100
—	6	—	6/7, 12/12	84.7
—	—	4	6/8, 9/11	78.9
150	6	—	5/8, 7/11	63.1
150	—	4	3/6, 7/10	62.5

*Treatment performed on Days 1, 4, 7, 13, and 15 after tumor surgery, which was performed on Day 12 after tumor was inoculated into the footpad of BALB/c mice.
†Bioassay evaluation carried out on Day 22 after tumor surgery.
‡Data from 2 experiments.

given daily (Days 1–9) at the dose of 30 mg/kg/injection while DX was administered every 3 days (Days 1, 4, and 7) after removal of the tumor. Neither ICRF-159 nor DX when given alone caused significant decrease in lung metastases, whereas the combined treatment did result in a significant reduction.

Table 7 shows the antimetastatic activity of DX plus ICRF-159 adjuvant chemotherapy injected at different times with respect to surgery. The experiments were carried out on BALB/c mice with MS-2 cells transplanted into footpads. Surgical amputation of the tumor-bearing leg was performed 13 days after implant, when 100% of control mice had already developed pulmonary metastases (24,27). The lung bioassay was carried out 7 days after the last treatment. ICRF-159 did not show any antimetastatic activity when given as a single agent at the dose of 30 or 50 mg/kg/injection ip either before or after surgery. Treatment with DX alone after surgery was inactive; treatment before, or both before and after, surgery significantly reduced the percent of mice with lung metastases (13 of 21 mice [62%], $P < 0.05$; and 14 of 24 [58%], $P < 0.05$, respectively). A synergistic effect of DX with ICRF-159 was observed on lung metastases when the treatment was carried out before the surgery (six of 23 mice [26%], $P < 0.01$ with ICRF-159 at 30 mg/kg/injection; and two of 24 [8.3%], $P < 0.01$ with ICRF-159 at 50 mg/kg/injection). The activity of DX plus ICRF-159 at 50 mg/kg/injection was statistically higher ($P < 0.01$) than the activity of DX administered alone. A synergistic effect, even if lower, was found also when the combined treatment

TABLE 6.—Activity of ICRF-159 administered daily in combination with DX administered on Days 1, 4, and 7 against MS-2 lung metastases*

Drugs	Dose (mg/kg/injection)	Lung metastases evaluation† No. of animals with tumor/ No. of mice inoculated	%
—	—	18/19	94.7
ICRF-159‡	30	12/19	66.6
DX§	4	9/20	45.0
DX§ + ICRF-159‡	4 + 30	5/17	29.4¶

*MS-2 tumor cells injected into the footpads of BALB/c mice.
†Bioassay evaluation carried out on Day 17 after tumor surgery.
‡Administered ip daily from Day 1 to Day 9 after tumor surgery.
§Administered iv on Days 1, 4, and 7 after tumor surgery.
¶$P < 0.01$ (Mussett's test between treated vs control mice).

TABLE 7.—Surgical-adjuvant DX + ICRF-159 combination chemotherapy against lung metastases of MS-2 sarcoma with respect to time of surgery*

Days of treatment†	Drug	Dose (mg/kg/injection)	Lung metastases‡ No. of animals with tumor/ No. of mice inoculated	%
—	—	—	25/26	96
Before surgery (Days 1,4,7)	ICRF-159	30	24/26	92
		50	24/25	96
	DX	4	13/21	62§
	DX + ICRF-159	4 + 30	6/23	26¶
		4 + 50	2/24	8.3¶**
—	—	—	23/24	96
After surgery (Days 15,18,21)	ICRF-159	30	23/24	96
		50	20/22	91
	DX	4	22/26	85
	DX + ICRF-159	4 + 30	21/24	87
		4 + 50	13/22	59¶
—	—	—	23/24	96
Before and after surgery (Days 1,4,7,15,18,21)	ICRF-159	30	24/26	92
		50	25/27	92
	DX	4	14/24	58¶
	DX + ICRF-159	4 + 30	7/20	35¶
		4 + 50	6/18	35¶

*Surgery carried out on Day 13 after tumor cells were implanted into the footpad of BALB/c mice.
†From tumor inoculation.
‡By bioassay, on Day 7 after the last treatment.
§$P < 0.05$.
¶$P < 0.01$ (Mussett's test between treated vs control mice).
**$P < 0.01$ (Mussett's test between group treated with DX + ICRF-159 vs group treated with DX alone).

was performed after the surgery (13 of 22 mice [59%], $P < 0.05$). When the combined treatment was performed both before and after surgery, the activity observed was not statistically higher than that observed after DX treatment alone.

Data from surgical-adjuvant chemotherapy experiments with DR plus ICRF-159 against lung metastases of MS-2 sarcoma are summarized in table 8. In these experiments, ICRF-159 administered alone at different times with respect to surgery did not show any antimetastatic activity. Treatment with DR alone before or after surgery was inactive; treatment before and after surgery significantly reduced the percent of mice with lung metastases (six of 14 mice [43%], $P < 0.01$).

Combined treatment performed after the surgery was not active. Combined treatment performed before the surgery was highly effective. While the single agents alone were not active or toxic, the combination treatment of ICRF-159 plus DR pro-

TABLE 8.—Effectiveness of surgical-adjuvant DR + ICRF-159 combination chemotherapy against lung metastases of MS-2 sarcoma with respect to time of surgery*

Days of treatment†	Drug	Dose (mg/kg/injection)	Lung metastases evaluation‡	
			No. of animals with tumor/ No. of mice inoculated	%
—	—	—	27/28	96
Before surgery (Days 1,4,7)	ICRF-159	30	11/12	92
		50	27/27	100
	DR	6	12/12	100
	DR + ICRF-159	6 + 30	11/13	84
		6 + 50	7/14	50§¶
	DR	8	9/12	75
	DR + ICRF-159	8 + 50	2/14	14**
	DR	10	ND (toxic)	—
	DR + ICRF-159	10 + 50	3/13	23**
—	—	—	13/13	100
After surgery (Days 16,18,21)	ICRF-159	30	11/12	92
		50	13/13	100
	DR	6	13/14	92
	DR + ICRF-159	6 + 30	13/14	92
		6 + 50	13/14	92
—	—	—	27/27	100
Before and after surgery (Days 1,4,7,16,18,21)	ICRF-159	30	15/15	100
		50	25/26	96
	DR	6	6/14	43**
	DR + ICRF-159	6 + 30	6/14	43**
		6 + 50	3/12	25**
	DR	8	3/6 (toxic)	50§
	DR + ICRF-159	8 + 50	2/10	20§
	DR	10	ND (toxic)	—
	DR + ICRF-159	10 + 50	4/11	36**

*Surgery carried out on Day 13 after MS-2 tumor cells were implanted into the footpads of BALB/c mice.
†From the tumor cell inoculation.
‡By bioassay, at Day 7 after the last treatment.
§$P < 0.05$ (Mussett's test between group treated with DR + ICRF-159 and group treated with DR alone).
¶$P < 0.05$.
**$P < 0.01$ (Mussett's test between treated vs control mice).

duced a significant reduction of the number of mice bearing lung metastases. The increased therapeutic effect was observed with ICRF-159 at the dose of 50 mg/kg/injection and DR at the doses of 6, 8, and 10 mg/kg/injection; with the highest DR dose, antagonism of toxicity by ICRF-159 was also seen. Similarly, in the groups treated with the combination of DR and ICRF-159 before and after the surgery, the percent of mice without metastases was significantly increased.

DISCUSSION

The data reported here partly confirm previous observations made by other authors (11,12). The injection of ICRF-159 ip into normal BALB/c mice after the iv administration of DR resulted in a marked decrease of the toxicity of the antibiotic. The best reduction of toxicity was observed with ICRF-159 at the dose of 75 mg/kg/injection. The protection decreased when the dose of ICRF-159 increased to 150 mg/kg/injection. In contrast, when DX was combined with ICRF-159, an increase in antibiotic toxicity was observed with high doses of the two agents; the one exception was the 10-mg/kg/injection dose of ICRF-159, which showed a slight protective effect. This decrease in DX toxicity is apparent only with low doses of ICRF-159, an effect which we plan to study further. These observations are partially in agreement with the data reported by Woodman et al (12) who found that ICRF-159 reduced the DR toxicity but did not increase the DX toxicity. They attributed the protection by ICRF-159 against DR toxicity (injected ip) to the anti-inflammatory activity of ICRF-159. This conclusion was based on the report of Lenaz and Di Marco (28) in which the authors stated that the primary cause of death in mice treated ip with toxic doses of DR was peritonitis.

Other hypotheses should be proposed with regard to the mechanisms of protection by ICRF-159 against the toxicity of DR injected iv, as observed in the experiments reported in this paper, and the increase of DX toxicity. Mhatre and Herman (29) reported that ICRF-159 given ip increased the tissue uptake and decreased the metabolic breakdown to aglycones of both DR and DX administered iv in hamsters; however, the modification of DR metabolism by ICRF-159 was not confirmed by Wang and Finch (30).

Like ICRF-159, EDTA has also been found to antagonize the toxicity of DR (13); such findings can suggest that Ca^{++} chelating agents could alter the intracellular distribution of DR, as this antibiotic is known to accumulate into the cell lysosomes (31), and Ca^{++} promotes the liberation of the lysosomal enzymes. On the contrary, DX is known to accumulate mainly in the cell nucleus, and EDTA was found to cause a marked increase of DX uptake by cells cultivated in vitro (32). This hypothesis, which could explain both the reduction of DR toxicity and the increase of DX toxicity remains to be confirmed in view of the fact that ICRF-159, even if synthesized with the aim of obtaining a chelating agent less polar than EDTA (33), did not affect the total ionized calcium concentration in hamster sera (34) and probably does not exert its antitumor and cytotoxic activity through chelating mechanisms since congeners of ICRF-159 free of chelating activity can exert an antitumor effect (33).

We think it is important to emphasize the increase in toxicity of DX by ICRF-159, in the perspective of future and present (19,20) clinical trials of combination therapy with DX and ICRF-159. In one such trial that drug combination was found to be more toxic to bone marrow than either drug alone (20).

The antitumor activity of ICRF-159 was tested on two experimental systems: MLV-M transplanted leukemia and MS-2 sarcoma. In mice bearing transplanted MLV-M leukemia, ICRF-159 administered alone at dose levels ranging from 70 to 150 mg/kg/injection did not significantly influence the tumor growth or the lifespan, nor was it synergistic with DR and DX at the tested doses. Only in the group treated on Day 1 with DR (12 mg/kg/injection) plus ICRF-159 (100 mg/kg/injection) after MLV-M tumor cell inoculum was an increase of the activity observed. The contrast with previously reported data (13,14) about a synergistic effect of ICRF-159 and DX against L1210 leukemia can be due to the different experimental tumors employed, or more probably to the different route of DX administration. In view of the fact that DX is administered to patients iv and ICRF-159 is administered to patients orally (35), we think it is important to carry out further studies in experimental tumors adopting these routes of treatment.

ICRF-159 administered independently at dose levels of 30 or 50 mg/kg/injection (five injections at 3–4-day intervals) in mice with MS-2 sarcoma did not result in a reduction of the tumor growth. Also, the combined treatment of ICRF-159 with DX at the doses tested did not result in a therapeutic synergism on MS-2 tumor growth, nor did it antagonize the antineoplastic action of DX; further studies are needed to verify whether, with other dose levels of the drugs, the combination therapy might be improved. On this point our results are not in accordance with those of other authors (12,15,33,36) who found antitumor activity of ICRF-159 against some tumor systems: Sarcoma 180, L1210 leukemia, Ca155, and Lewis lung carcinoma.

In our studies a marked inhibition of tumor growth and increase in lifespan were observed in the groups of mice treated with the association of a toxic dose of DR (10 mg/kg/injection) plus 50 mg/kg/injection of ICRF-159. The reduction of DR toxicity by ICRF-159 resulted in a better therapeutic ratio in the MS-2 system than that achieved by DX treatment alone or combined with ICRF-159.

The protective effect of ICRF-159 is of great interest because it reduces both cardiac (8,9), marrow, and gut toxicity, thus allowing an increase in the dose of anthracycline and consequently an improved antineoplastic activity.

In our studies we did not find any evidence of antimetastatic activity of ICRF-159 when administered alone, either at high or low doses. Such activity has been reported by various authors in several animal systems (17,18,37,38). Pimm and Baldwin's results (39) showing the inactivity of ICRF-159 on pulmonary metastases arising from the subcutaneous epithelioma Spl are in agreement with our data. It is possible that the antitumor and antimetastatic activities of ICRF-159 depend on the experimental tumor system adopted, as well as on the route and schedule of treatment.

Actually, the half-life of ICRF-159 in all of the species examined is fairly short (40,41). Therefore, the schedule of ICRF-159 treatment is very important. We had administered the chelating agent every 3 days or every day for 9 days. In the latter case, the treatment caused a greater reduction in the percent of mice with pulmonary metastases than in the experimental group treated with the former schedule. Experiments are in progress to study the activity of ICRF-159 administered alone or in combination with DX or DR many times a day.

We have examined the effectiveness of surgical adjuvant combination chemotherapy with DR or

DX plus ICRF-159 supplied at different times with respect to surgery. ICRF-159 did not show any antimetastatic activity given at any time as a single agent. The combined chemotherapy performed early in the course of the disease and before the surgery resulted in a "true" synergistic effect which was statistically significant. These observations can have various explanations. One is that ICRF-159 has an antimetastatic effect, which is not due to direct killing of metastatic cells but to other mechanisms, such as changes in the vasculature that prevent the entry of tumor cells from the primary tumor into the circulation (42), an inhibition of blood coagulation, or an antiproteolytic effect. However, these effects can be combined with the reduction of DR toxicity and consequently improved antineoplastic activity or with the possible increased uptake of DX and consequent increase of DX antitumor and toxic activity. In fact, we have observed a higher antimetastatic activity in the groups treated with a combination of toxic doses of DR and ICRF-159 than in the groups treated with tolerated doses of the antibiotic.

Adjuvant chemotherapy with DR plus ICRF-159 after the surgery was slightly effective (DR was inactive). This probably occurred because of the huge number of metastatic foci at the start of chemotherapeutic treatment.

In conclusion, these studies indicate that chemotherapeutic drugs having an antimetastatic effect in some experimental systems may not be generally effective in other systems. These data also show the superiority of combined-modality treatments against lung metastases. In particular, the synergism between active agents (the anthracyclines) and agents with little or no activity (ICRF-159) may be particularly relevant for combination clinical trials.

Furthermore, the observation that the reduction in the toxicity of antitumor drugs can be achieved by administration of chemicals with antidotal activity is of significance. The protection against toxicity (marrow, gastrointestinal, myocardial, etc) may allow an increase in drug dose with a consequent increase in antineoplastic activity (13).

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Improved Antitumor Activity of Basic Amino Acid and Dipeptide Derivatives of Daunorubicin on EL4 Leukemia Cells in Mice[1,2]

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SUMMARY

Over thirty amino acid and peptide derivatives of the antitumor drug daunorubicin (DM) were tested for their potency to inhibit EL4 leukemia cell growth in mice. The therapeutic effect of the basic amino acids lysine, arginine, ornithine, and 2,4-diaminobutyric acid coupled to the amino group of the DM moiety proved superior to that of the parent drug. The derivatized amino acids and their di- or tripeptides are significantly less toxic than DM, which enabled their administration at much higher doses. Seventy percent to 80% of tumor-bearing C57BL/6 mice were cured by multidose treatment with diaminobutyryl-DM, which was found to be the most efficient derivative.

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Daunorubicin (DM) is one of the anthracycline derivatives that has been used as an antitumor drug, particularly in the treatment of various leukemias. The antineoplastic activity of the drug relates to its ability to intercalate with DNA, both via hydrophobic interactions between the base pair of the DNA and the aglycon and by binding of the amino group of the sugar residue in the drug to the phosphate moiety of DNA (1).

The use of DM in chemotherapy has, however, been hampered by its toxic effects, manifested in both the heart muscle and the bone marrow system. There have been several attempts to modify the drug to diminish its toxic effects (2–5), and blocking of the free amino groups of the sugar moiety of the drug has been effective (1,2,6,7). However, such modification of DM decreased the binding of the drug to nucleic acids and consequently reduced its antineoplastic effects.

We have taken the approach of converting DM to several nontoxic derivatives by binding amino acids or peptides to the amino group of the drug. Such treatment did not prevent the intercalation of the modified drug with DNA. It was found that the antitumor activity of the peptide derivatives of DM in vitro decreased proportionally to the length of the peptide chain (6,8) but could be significantly restored by enzymatic cleavage of the peptide moiety from the derivatized drug (8). Assuming that a similar reaction of the drug could occur in the body by proteolytic hydrolysis of the attached peptides, these derivatives were tested on experimental tumors in vivo.

MATERIALS AND METHODS

Commercial DM·HCl was purchased from Specia, France; 9-fluorenylmethylchloroformate was from Research Products, Rehovot, Israel; *N*-carboxyanhydrides (NCAs) of the various amino acids, prepared by the Fuchs-Farthing method (9) were kindly provided by I. Jacobson of this laboratory. NCA of arginine was prepared according to Hirschmann et al (10).

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Cells

The chemotherapeutic activity of the derivatized DM was studied on 4–5-week-old, male C57BL/6 mice. Syngeneic EL4 lymphoma tumor cells were inoculated ip into mice weighing 15–18 g. The drugs were administered ip beginning 2 days after inoculation of the cells, followed by daily injections thereafter. Evaluation of the chemotherapeutic effects was based on the increase in lifespan, on complete depression of tumor growth in animals surviving 90 days, and on decreased toxic reactions in tested mice.

Amino Acid and Peptide Derivatives of DM

Amino acid derivatives of DM were prepared by reacting succinimide esters of *N*-fluorenylmethoxycarbonyl (Fmoc) amino acids with DM. Fmoc amino acids were prepared following the procedure of Carpino and Han (11). As an example, the preparation of *N*-lysyl-DM is given below.

N^{α}, N^{ϵ}, di-Fmoc-lysine (290 mg, 0.35 mmol) and *N*-hydroxysuccinimide (44 mg, 0.38 mmol) were dissolved in *N*,*N*-dimethylformamide (DMF) (3 ml). *N*,*N*′-dicyclohexylcarbodiimide (DCC) (82 mg, 0.4 mmol) dissolved in DMF (0.5 ml) was added to this solution and left in the cold overnight. The precipitated *N*,*N*′-dicyclohexylurea was filtered off, and the solution containing di-Fmoc-lysine succinimide ester was used without further workup.

DM·HCl (60 mg, 106 μmols) dissolved in a mixture of 1:1 dioxane: 0.1 M sodium bicarbonate (4 ml), pH 8.5, was reacted with excess of di-Fmoc-lysine succinimide ester dissolved in DMF. The reaction was completed within 5–10 minutes and was followed by TLC in chloroform:MeOH:H_2O 13:6:1 (R_f of DM, 0.6; di-Fmoc-Lys-DM moves with the front). The di-Fmoc-Lys-DM thus obtained was extracted into chloroform, which was then washed with 0.1 M sodium bicarbonate (pH 8–8.5), water, and dilute HCl (pH 2–3) and was dried over anhydrous sodium sulphate. One milliliter of 1 N sodium methylate in methanol was then added to the chloroformic solution for 1–2 minutes to remove Fmoc blocking groups. The resulting Lys-DM was immediately extracted into water and acidified with HCl to pH 7–8. The aqueous phase was washed several times with chloroform and was lyophilized.

Peptide derivatives of DM were prepared as described before (8) with a slight modification. The free base of DM was obtained by dissolving DM·HCl in 0.01 M sodium bicarbonate and extracting the free base with chloroform. The chloroformic solution was dried over sodium sulfate and the solvent was removed in vacuo. The dry free base of DM was dissolved in dry DMF and reacted with NCA of the appropriate amino acid as described (8). A scheme of derivatized DM is shown in figure 1.

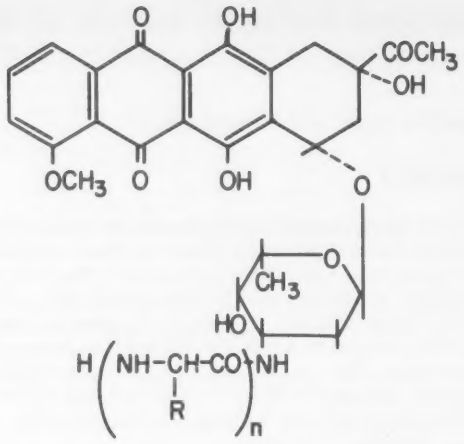

FIGURE 1.—Structure of amino acid or peptide derivative of DM.

RESULTS AND DISCUSSION

When C57BL/6 mice were inoculated ip with EL4 lymphoma cells, followed by a series of DM injections, no satisfactory therapeutic effect could be achieved (table 1). It was apparent that the unmodified drug, at various schedules of drug administration, failed to inhibit tumor growth in mice inoculated with EL4 cells. As shown in table 1, the optimal combination of four daily 30-μg injections inhibited tumor growth in only 25% of the treated mice that survived the toxic effects of DM. On the other hand, injection of suitable doses of several amino acid and peptide derivatives of DM proved less toxic than the parent drug and brought about a significant increase in the lifespan of tumor-bearing mice; in most cases it even resulted in complete inhibition of tumor growth. It is evident from table 2 that the acidic amino acid derivative aspartyl-DM was nontoxic when administered even at high doses (four daily injections of 1500 μg or a single injection of 3000 μg/mouse as compared with a fatal single dose of 80 μg of DM). Similarly, the heterodipeptide derivative Asp-Lys-DM was found to be significantly less toxic than the dilysyl derivative. In general, the basic amino acids lysine, arginine, ornithine, and diaminobutyric acid (A_2bu) derivatized to DM show higher toxicity than the acidic ones; however, their toxicity diminishes with the de-

TABLE 1.—Effect of repeated treatments with DM on EL4 lymphoma inoculated ip*

Dose (μg/mouse)	Median survival time† (days)	No. of mice surviving > 90 days/ No. of mice treated	No. of toxic deaths
Control	13	0/40	—
80 × 1	17	0/8	8
60 × 1	18	0/8	6
40 × 1	19	0/8	2
50 × 2	19	0/12	3
40 × 4	18	0/12	6
30 × 4	25	4/16	5
20 × 4	17.5	0/12	0
15 × 6	19	0/12	0
25 × 4	14.5	0/12	3
25 × 5	24.5	2/16	4
20 × 5	17	2/16	2
20 × 6	15	0/12	6

*10^6 EL4 lymphoma cells were inoculated ip into 4-wk-old C57BL/6 males. Drug administration started 2 days later.
†Refers to group of mice that did not survive.

crease in the number of methylene groups in the side chain. Thus, the induced toxic effects decrease when the lysine derivative of DM is replaced by the ornithine derivative, and they decrease even more significantly when the A_2bu and diaminopropionic acid (A_2pr) derivatives are used. In parallel, we have carried out experiments in C57BL/6 mice to determine the LD50 value of Orn_2-DM compared with the parent drug. It was found that, whereas a single ip inoculation of 78.9 μg of DM killed 50% of the tested mice, 1708.6 μg of Orn_2-DM was required to give the same lethal effect.

It is suggestive that the hydrophobic amino acid derivatives such as alanine and leucine are more toxic than the hydrophilic amino acid derivatives such as the basic and acidic derivatives (table 2). The strongly hydrophobic compounds phenylalanyl-DM and tyrosyl-DM are only slightly soluble in water and therefore could not be used for inoculation into mice. These derivatives could however become soluble on introduction of hydroxyl groups into the aromatic ring to render them more hydrophilic, as is evident from the enhanced solubility of the hydroxylation product of phenylalanine, dihydroxyphenylalanine (DOPA), coupled to DM. The diminishing toxic effect caused by introducing hydrophilic groups into the derivatized molecule can be further demonstrated by comparing DOPA-DM to Leu-DM. While all tested mice died of excessive toxic effects when they were injected eight times with 100 μg of Leu-DM, they survived the toxic effects of eight injections of 250 μg of DOPA-DM.

It was apparent that reduced toxic effects of a given derivative of DM could not always be correlated with improved therapeutic efficacy. Thus, although aspartyl- and prolyl-DM were found to be nontoxic at the concentrations used in these experiments, they were only poorly effective in eradication of the propagating tumor cells.

The most promising antitumor effect was found with the basic amino acids lysine, ornithine, and A_2bu and their dipeptide derivatives. Tumor growth was suppressed in up to 80% of the tumor-bearing mice that survived at least 90 days, at which time the experiment was terminated. As shown in table 2, the most efficient antitumor effect in our experiments was obtained with A_2bu-DM, which completely cured almost 80% of the tested mice. This derivative was also of low toxicity, and eight 650-μg injections per mouse still did not cause any obvious toxic damage.

Interestingly, the shortest diamino acid derivative, A_2pr-DM, though inoculated at high doses due to its very low toxicity, did not have satisfactory curative effects, in sharp contrast to A_2bu-DM, which differs in only one methylene group. This could reflect the fact that the pKa value for the α-amino group of A_2pr (pKa = 6.7) is much lower than that of lysine, ornithine, or A_2bu, which have pKa values of 9.2, 8.7, and 8.3, respectively. Since the pKa values of α-amino groups of peptides or amides of amino acids are even lower than those of the corresponding free amino acids, the α-amino group of A_2pr-DM is practically un-ionized under physiologic conditions. This derivative therefore behaves as a hydrophobic compound, differing from the other diamino acid derivatives that possess two ionized amino groups.

TABLE 2.—Effect of repeated treatment with amino acid and peptide derivatives of DM*

Drug	Dose (μg/mouse)†	Median survival time‡ (days)	No. of mice surviving > 90 days/No. of mice treated	No. of toxic deaths
Control	—	13	0/40	—
Lys-DM	250 × 8	26	11/16	2
Lys_2-DM	250 × 8	24	6/8	1
Lys_3-DM	250 × 8	25	6/8	1
D-Lys-DM	300 × 8	14	0/8	0
D-Lys-DM	600 × 4	12	0/4	4
Orn-DM	300 × 8	30	2/12	0
Orn-DM	600 × 4	11	0/4	4
Orn_2-DM	300 × 8	29	9/12	1
Orn_5-DM	300 × 8	38	3/12	1
Arg-DM	250 × 7	35	3/4	0
Arg-DM	400 × 6	19	3/4	1
A_2bu-DM	650 × 8	30	11/14	0
A_2pr-DM	600 × 7	19	0/4	0
A_2pr-DM	750 × 8	18	0/4	1
His-DM	250 × 8	25	3/8	0
Leu-DM	100 × 6	19	0/4	0
Leu-DM	100 × 8	15	0/4	4
DOPA-DM	250 × 8	28	3/8	0
Pro-DM	600 × 8	26	0/4	0
Asp-DM	1500 × 4	21	0/4	0
Asp-DM	3000 × 1	23	1/4	0
Asp-Lys-DM	600 × 8	31	6/8	0

*10^6 EL4 lymphoma cells were inoculated ip into 4-wk-old C57BL/6 males. Drug administration started 2 days later.

†Dose weight is expressed in terms of DM in the molecule.

‡Refers to groups of mice that did not survive.

The variations in toxicity and therapeutic index of the various derivatives may be attributed to several parameters such as the differences in tissue distribution of these derivatives and the rate of their uptake by cells. Deprez-de-Campeneere et al (12) have shown that the tissue distribution of detorubicin (14-diethoxyacetoxydaunorubicin) differs significantly from that of doxorubicin and DM, and it was indeed suggested that variations in chemical structure could alter the distribution properties of drugs (3). It is possible that the concentration of some of the amino acid and peptide derivatives of DM in heart tissues is decreased compared with the parent drug, as was shown for L-leucyl-DM and L-leucyl-doxorubicin (13). Other factors which may affect the selectivity of these derivatized drugs are the differences in their kinetics of transport into cells and in their intracellular localization (14). Using fluorescent microscopy, we have found drastic variations in the penetration of various amino acid derivatives of DM (8) when negatively charged DM derivatives like succinyl-DM did not penetrate into the cells to any significant extent and accumulated on the cell surface.

Differences in the net charge of the derivatized drug are also important factors in assessing its biologic properties. Bachur et al (15) and Di Marco et al (16) proposed that the altered polarity of a number of anthracycline antibiotics contributes to the differences in their therapeutic indices and in vivo activities.

Goldman et al (17) have measured the partition coefficient of DM, doxorubicin, and some of their derivatives in octanol and buffer and have suggested that such biphasic partition could serve as an indication for possible drug-membrane interaction. Such measurements have indicated that doxorubicin is less lipophilic than DM. We have measured the partition coefficients of DM and some of its amino acid derivatives in octanol and phosphate buffer, pH 7.4, and they were found to be 3.9, 0.02, 0.37, and 5.2 for DM, Lys-DM, A_2bu-DM, and A_2pr-DM, respectively. The very low lipophilicity of Lys-DM and A_2bu-DM is compatible with their reduced toxic effects inasmuch as high lipophilicity was implicated in the cardiac toxicity of the drugs (18,19). In contrast to what was reported by Baurain et al (20) for L1210 leukemia cells in DBA/2 mice, we did

not find an improved therapeutic effect of L-leucyl-DM or L-alanyl-DM compared with DM. Whereas these two amino acid derivatives of the drug were reported to be only four to six times less toxic than DM (20), we have found that derivatives of the basic amino acids ornithine and A_2bu were 22- to 30-fold less toxic than the parent drug in our system.

The amino acid and peptide derivatives of DM may act as "slow-release" drugs. It is assumed that proteolytic cleavage of the derivatized moiety that occurs in vivo gradually reactivates the drug. The differences in the therapeutic indices of the various derivatives could relate also to the specific proteolytic enzymes that would react on the different amino acids. The fact that D-Lys-DM exhibits a very poor curative effect on leukemic mice, in contrast to L-Lys-DM, supports an involvement of a slow-release drug mechanism. Such an assumption, however, probably does not apply to A_2bu-DM, which is less likely to be cleaved enzymatically, and the improved curative effect could be due to a more efficient distribution and penetration of the uncleavable derivative into target tissues and cells.

The data presented in this report suggest that amino acid and peptide derivatization of existing toxic antitumor drugs may render such drugs less damaging and more efficient chemotherapeutic agents. Current experiments in our laboratory make use of the various derivatives of DM to treat different types of ascitic and solid tumors. Preliminary studies with YAC lymphoma cells in A/J mice and with P388 cells in DBA/2 mice suggest that the enhanced antitumor efficacy of some of the tested derivatives of DM also applies to other tumor systems.

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Action of Demecolcine (Colcemid) in the Murine Sarcoma 180 Tumor[1,2]

Tadao Nomura and John E. Plager[3,*]

SUMMARY

Iv infusions of demecolcine (Colcemid) were established in mice bearing the Sarcoma 180 tumor. During demecolcine infusion at a rate of 6 μg/hr, the tumor labeling index following ^{3}H-thymidine administration and the mean grain count per labeled cell did not differ significantly from control values. Cells in anaphase and telophase disappeared from the tumor sections, and the net number of cells in the earlier stages of mitosis increased for 20 hrs. The rate of mitotic accumulation during the initial 8 hrs of demecolcine infusion was about one third of what would be expected from the known cell cycle values for this tumor. This reduced rate of accumulation does not appear to result from death of mitotic cells, escape of cells from metaphase blockade, or S-phase arrest secondary to inhibition of DNA synthesis; it is suggested that the reduced accumulation rate is secondary to demecolcine damage to interphase cells. Tumor cells that have been gathered by 4-hr demecolcine infusions may be released in synchronous fashion following the end of the infusion. A small proportion of these cells will recycle synchronously during the following mitotic period.

[Cancer Treat Rep 65:283–290, 1981]

Many of the biologic effects of colchicine, including its antimitotic action, are the result of microtubule disruption (1,2). Colchicine is known to rapidly penetrate into cells and bind to a protein that is probably a microtubule subunit (1). The binding of tubulin with colchicine has the properties of a biomolecular reaction (2); binding is noncovalent, colchicine is not chemically altered (2), and the rate of reversible dissociation of the colchicine-tubulin complex is quite slow (1,3). Since tubulin appears to be synthesized for the following mitotic period primarily in late S phase and in G_2 (4), a short latent period between colchicine administration and metaphase blockade would be anticipated, and this has been found (5). The administration of colchicine at minimal concentrations sufficient to inhibit mitosis does not usually affect the rate of DNA, RNA, or protein synthesis, and the duration of the cell cycle intervals other than mitosis are also unchanged (1).

Demecolcine (Colcemid) is a colchicine derivative that while sharing many of colchicine's biologic properties (5–9), has been reported to be appreciably less cytotoxic and more effective in inhibiting formation of the mitotic spindle (9–12). In addition, tissue culture cells arrested in metaphase by demecolcine have been reported to complete mitosis when demecolcine is removed from the medium, and to undergo a second, synchronous division many hours later (5,11).

Demecolcine has been empirically used in the Department of Medicine B, Roswell Park Memorial Institute, in the treatment of the more aggressive malignant lymphomas and chronic myelogenous leukemia in blastic transformation. However, little information is presently available in an animal tumor

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system about the kinetics of action of this drug, its reversibility, and its possible activity as a synchronizing agent. In addition, we wished to utilize demecolcine as a tumoricidal agent in the murine Sarcoma 180 (S180) tumor previously synchronized by pulsed hydroxyurea infusion (14), but we had little information about the onset and duration of its action. These objectives encouraged the present study of the kinetics of demecolcine action on the murine S180 tumor.

MATERIALS AND METHODS

Male, white, Swiss Ha/Icr mice, 10–13 weeks old and weighing between 25 and 30 g, were used. The mouse sarcoma, S180, was maintained by passage at 10-day intervals. In the present experiments, 5-mg tumor fragments were implanted by trocar at two subcutaneous sites, and the mice were studied 10 days later. At this time the tumor at each site weighed between 500 and 1000 mg.

The technique for establishing and maintaining an iv infusion in the tail vein of unanesthetized mice has been published (13,14). All experimental procedures were started 18–24 hrs after the beginning of the infusion. Throughout the experiments the mice remained undisturbed in their cages, with available food and the prevalent day-night cycle.

Demecolcine was purchased from Ciba Pharmaceutical Co, Summit, NJ, and was stored in a 1-mg/ml stock solution at 4°C (10 mg of demecolcine, 0.67 ml of 95% ethanol, 0.96 ml of propylene glycol, 0.015 g of $Na_2HPO_4 \cdot 7H_2O$, 0.0133 g of $NaH_2PO_4 \cdot H_2O$, distilled water to a volume of 10 ml). The stock solution was diluted to the desired concentration with Ringer's solution immediately before use.

[*Methyl*-^{3}H]thymidine (6.7 millicuries/mM) was purchased from New England Nuclear Corp, Boston, MA; 24 microcuries (in 0.1 ml of Ringer's solution) was injected into the iv infusion at the time interval indicated in each experiment. Mice were killed by cervical dislocation, the tumors were fixed in neutral formalin, and slides were prepared. For autoradiographic study, the slides were dipped in Kodak NTB-2 emulsion diluted 1:1 with water, and then exposed in a desiccant box at 5°C for 2 weeks. They were developed in Kodak D-19, rinsed in water, and cleared in Kodak rapid fixer. Slides were stained with Giemsa.

One grain per cell was the maximal control count determined from autoradiography of tumor obtained from animals that had not been given radioactivity. A cell was therefore considered labeled if two or more grains were superimposed. The percent labeled mitoses was determined from ≥ 200 mitotic cells/tumor. The number of mitoses per 100 cells (MI), the number of labeled mitoses per 100 cells (LMI), and the number of labeled cells per 100 cells (LI) were determined by evaluating 1000 cells from each of three inner and three outer portions of three separate sections of the tumor (a total of 18,000 cells counted). The mean grain count data were derived from 200 consecutive labeled cells chosen at random from the tissue sections. The proportion of cells in each of the mitotic phases was evaluated from 200 randomly chosen mitotic figures.

In this report, numeric values are presented as the mean ± SD; data for each of these calculations are derived from five to ten different tumors. The value from each tumor was treated as a single entry for statistical purposes.

RESULTS

Mitotic Accumulation Following a Single Demecolcine Pulse

The effect of a single pulse of demecolcine on mitotic accumulation in the S180 tumor was studied in initial experiments. Three concentrations of demecolcine were given iv: 0.5, 1.0, or 2.0 μg/g of body weight. The mice were killed during the 16 hrs following demecolcine administration, and the tumor MI was determined (fig 1). Significant mitotic accumulation was noted 1 hr after demecolcine administration ($P < 0.01$). At the 1- and 2-hr intervals, the rate of mitotic accumulation was similar for all three demecolcine concentrations and was similar for the two higher concentrations at the 4-hr intervals. The maximal mitotic rate was delayed with increasing demecolcine dose; an MI of 12.8 was seen 6 hrs after the 2.0-μg/g dose. This represents a net 6-hr accumulation rate of 2.1% ± 0.3% cells/hr at this highest dose. During the 10–12-hr interval following demecolcine administration, the MI returned to control levels.

Mitotic Accumulation With Different Rates of Demecolcine Infusion

Experiments were designed to determine the optimal rate of demecolcine infusion for metaphase blockade. Several concentrations of demecolcine were infused for 14 hrs, at a constant rate, into tumor-bearing animals, and the MI was determined in these tumors after the infusion (fig 2). The MI increased with increasing infusion rates to 6 μg/hr and did not increase further at a demecolcine infusion rate of 9 μg/hr. The MI was approximately 20% following 14 hrs of infusion at the two higher rates.

The ratio of anaphase plus telophase cells to pro-

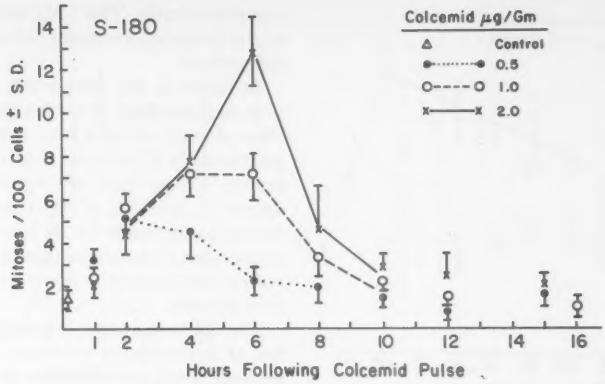

FIGURE 1.—MI/100 S180 tumor cells following the administration of a single iv pulse of demecolcine. Dose of demecolcine was 0.5, 1.0, or 2.0 μg/g of mouse weight.

phase plus metaphase cells was also determined in these experiments in order to estimate the infusion concentration at which demecolcine blockade is complete (fig 3). With increasing infusion rates between 0.6 and 6 μg/hr, there was a gradual increase in blockade. Cells in anaphase and telophase were seen following infusions at ≤ 3 μg/hr. A rate of 6 μg/hr resulted in complete metaphase blockade; no cells in anaphase or telophase were seen in these tumor sections. This infusion rate was therefore used in experiments in which complete blockade was desired.

Prolonged Infusions of Blocking Concentrations of Demecolcine

Tritiated thymidine was administered iv immediately prior to the beginning of a demecolcine infusion at 6 μg/hr into tumor-bearing mice. The mice were killed at intervals during the subsequent 24-hr infusion, and the tumor MI and LMI (fig 4), the LI, and the proportion of all mitoses that were labeled were determined (fig 5).

The MI increased during the initial 20 hrs of infusion and then dropped abruptly (fig 4). Following

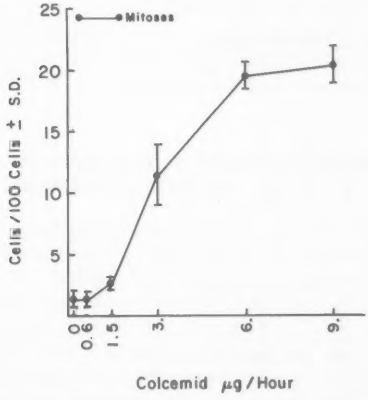

FIGURE 2.—MI/100 S180 tumor cells following 14 hrs of demecolcine infusion. Demecolcine was infused iv at the indicated rates.

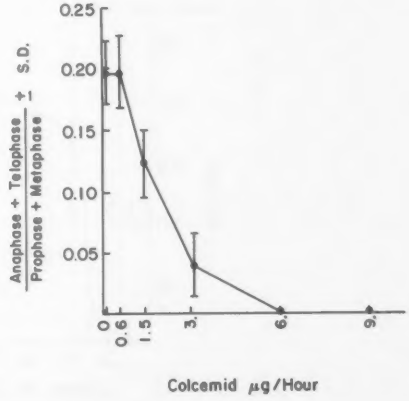

FIGURE 3.—Ratio of anaphase + telophase to prophase + metaphase cells in the S180 tumor, following a 14-hr iv infusion of demecolcine at the indicated rates.

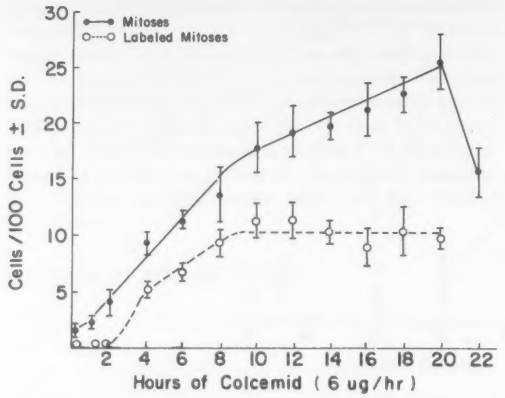

FIGURE 4.—MI and LMI/100 S180 tumor cells during a 22-hr iv infusion of demecolcine at 6 μg/hr. Tritiated thymidine was given iv at the beginning of the demecolcine infusion.

24 hrs of demecolcine infusion, tumor sections showed dissolution of the nuclear membrane with karyolysis, complete loss of cellular details, and cytolysis, with only necrotic cell debris remaining. Between the 1- and 8-hr interval, the increase in MI was 1.7%/hr (r = 0.965), and from 10–20 hrs, MI increased 0.7%/hr (r = 0.978).

Since the duration of the G_2 and S cell cycle phases (T_{G2} and T_S) in the S180 tumor is 2.3 and 8.3 hrs, respectively (14), the number of labeled cells entering into mitosis would be expected to be insignificant for the initial 2 hrs and then to increase during the subsequent 8–9 hrs. This was confirmed experimentally. The LMI increased to a maximum at the 10-hr interval and did not change appreciably thereafter.

In figure 5, the proportion of labeled mitotic figures is presented at each time interval. This curve rises sharply after 2 hrs, remains elevated for approximately 8 hrs, and falls following the 12-hr interval. These data are again consistent with the known T_S and T_{G2} of this tumor. Since the MI continues to increase for 20 hrs (fig 4), the fall in the proportion of labeled mitoses after the 12-hr interval predominantly reflects unlabeled cells entering into mitosis.

The LI remains at control levels for the initial 4 hrs of demecolcine infusion, after which it falls to a lower level and remains at this level during the 10–20-hr period. All cells found to be labeled after the 10-hr interval are in mitosis.

Rate of Metaphase Blockade and Release

Demecolcine was infused into tumor-bearing mice at 6 μg/hr for 4 hrs. The ratio of tumor anaphase plus telophase to prophase plus metaphase cells was studied during the infusion period and for the subsequent 10 hrs (fig 6). There was no significant effect on this ratio 1 hr after the beginning of the demecolcine infusion, but the ratio was clearly decreased by 2 hrs, and by 4 hrs anaphase and telophase cells were no longer seen. One hour after the end of the demecolcine infusion, anaphase and telophase cells were still absent, but they reappeared 2 hrs after the end of the infusion and were in normal ratio at subsequent time intervals.

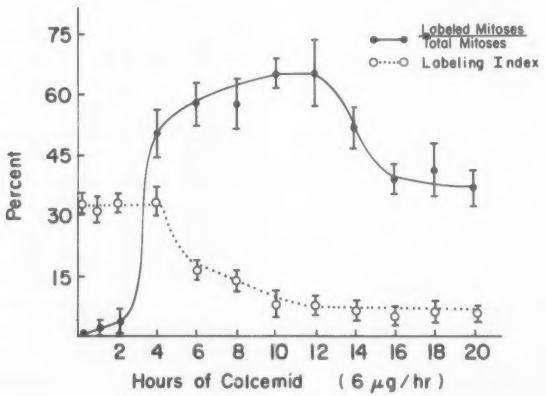

FIGURE 5.—% of labeled mitotic figures and the tumor LI during a 20-hr iv demecolcine infusion at 6 μg/hr. Tritiated thymidine was given iv at the beginning of the demecolcine infusion.

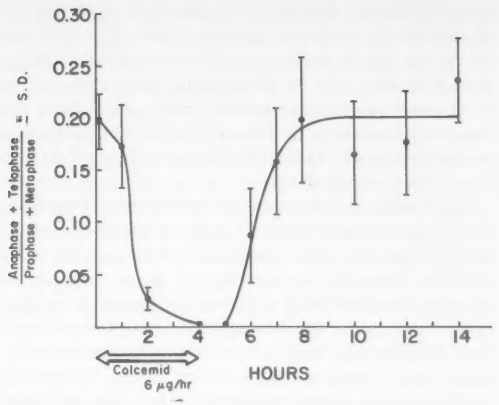

FIGURE 6.—Ratio of anaphase + telophase to prophase + metaphase cells in the S180 tumor during 4 hrs of iv demecolcine infusion at 6 μg/hr and during the 10 hrs following the end of demecolcine administration.

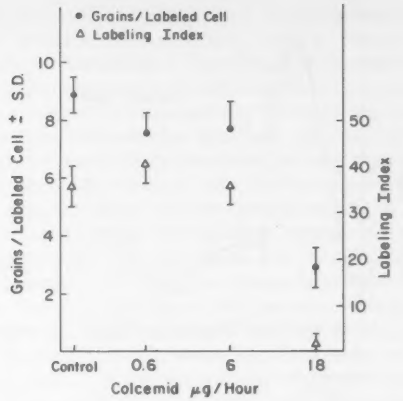

FIGURE 7.—No. of grains/labeled cell and the LI in the S180 tumor following iv demecolcine infusion at 3 different concentrations. Tritiated thymidine was given iv 3.5 hrs after the beginning of the demecolcine infusion, and the mice were killed at 4 hrs.

Effect of Different Demecolcine Infusion Rates on Cell Labeling by ^{3}H-Thymidine

Demecolcine was infused at rates of 0.6, 6, or 18 μg/hr for 4 hrs, at which time the animals were killed. Tritiated thymidine was injected iv 0.5 hr prior to death. Tumor sections were autoradiographed; the number of grains per labeled cell and the LI are plotted in figure 7 for each rate of demecolcine infusion. A significant effect on thymidine incorporation or the tumor LI was seen only at 18 μg/hr; at this infusion rate both of these were significantly inhibited ($P < 0.01$ for both).

The MI was also determined in this experiment for each rate of demecolcine infusion (fig 8). While the MI increased with increasing administration rates to 6 μg/hr, a rate of 18 μg/hr produced a significant drop ($P < 0.01$) in net mitotic accumulation.

Tumor Synchronization Following Release From Demecolcine Blockade

Demecolcine was infused at 6 μg/hr for 4 hrs into tumor-bearing mice. The tumor MI was measured during the infusion and during the subsequent 24 hrs (fig 9). The MI increased during the 4-hr infusion and remained elevated through the 7-hr interval, following which it fell abruptly. This sharp fall in the MI suggests synchronous release of cells from demecolcine blockade into the subsequent growth phases. The previous observation (fig 4) that the MI will continue to rise if the demecolcine infusion is prolonged beyond 8 hrs also suggests that this 8-hr drop in MI represents the release of metaphase-arrested cells.

A second peak of mitotic cells is seen at the 20-hr interval. The MI at 20 hrs is significantly greater than values at the 18- or 22-hr intervals ($P < 0.05$ and $P < 0.02$, respectively).

DISCUSSION

When demecolcine is given in a single pulse, the subsequent mitotic accumulation attains a maximal value for a relatively brief time, and the tumor soon

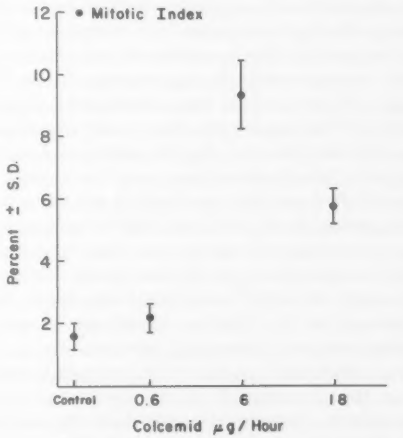

FIGURE 8.—MI of the S180 tumor following 4 hrs of iv demecolcine infusion at 0.6, 6, or 18 μg/hr.

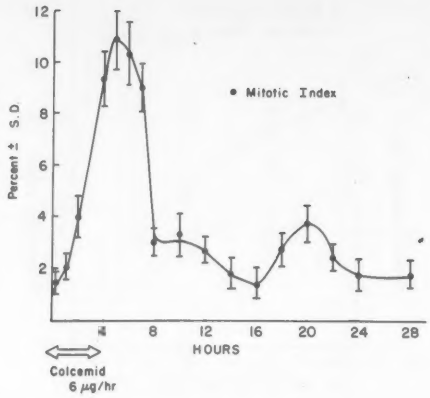

FIGURE 9.—MI of the S180 tumor during 4 hrs of iv demecolcine infusion at 6 μg/hr and during the subsequent 24 hrs.

escapes from blockade. The blockade may be sustained by either repeated pulses of demecolcine or by continuous iv infusion. The latter choice appears preferable.

Many of the biologic effects of colchicine, including its antimitotic action, result from microtubule disruption (1,2). However, it also exerts effects that are not secondary to disruption of microtubules, eg, nucleoside transport inhibition (2). Colchicine will inhibit ^{3}H-thymidine incorporation during incubation with guinea pig skin slices (16). In addition, studies utilizing cultured human lymphocytes show decreased ^{3}H-thymidine labeling following colchicine addition; at high colchicine concentrations the MI is also reduced, suggesting inhibition of cell progression during interphase (17). Since demecolcine does not inhibit ^{3}H-thymidine incorporation when added in concentrations equivalent of that of colchicine (16), it appears that colchicine has greater activity in this respect. In the present experiments, demecolcine infusion at 6 μg/hr produces a complete metaphase blockade without any discernible inhibition of ^{3}H-thymidine labeling of the S180 tumor. During infusions at this rate, tumor anaphase and telophase cells are no longer seen and the MI reaches maximal values. Release from demecolcine blockade at this infusion rate is fairly rapid; following the end of the infusion, significant numbers of anaphase and telophase cells are seen within 2 hrs.

There does not appear to be appreciable alteration in the duration of the G_2 or S phase during demecolcine administration. In both figures 4 and 5, the labeled-mitoses curves rise abruptly during the 2–4-hr interval following tritiated thymidine administration; this reflects the G_2-S junction and is consistent with the known 2.3-hr T_{G2}. Furthermore, in figure 5, the percent of labeled mitoses falls following the 12-hr interval; this reflects the G_1-S junction. The duration of the S phase from this curve is consistent with an 8.3-hr T_S (14), although an accurate calculation of this value is not possible from the present data.

Colchicine and demecolcine are known to produce metaphase arrest (1–5). It may therefore be anticipated that the lethal action of demecolcine is related to this mitotic activity. It is an unexpected observation that S180 tumor cells blocked in mitosis do not appear to be damaged for many hours, and that during this time nonmitotic cells are being injured and removed. The LI of figure 5 remains within normal range during the first 4 hrs of demecolcine infusion and then falls during the next 6 hrs to a level at which essentially all labeled cells are in mitosis. During the 10–20-hr interval this LI does not change significantly and the labeled mitosis level in figure 4 also remains constant. These observations suggest that cells blocked in mitotic phase are not particularly sensitive to demecolcine damage during this time period. It is, therefore, likely that the 4–10-hr fall in LI (figure 5) reflects damage and removal of labeled nonmitotic cells. In addition, since this tumor has a growth fraction of one and a cell cycle duration of 19.6 hrs (14), during complete metaphase blockade approximately 5% of cells should enter into mitosis per hour, and after 20 hrs all cells should be in mitosis. However, the net entry of cells into mitosis during the first 8 hrs of demecolcine infusion at 6 μg/hr is only 1.7%/hr, and at later infusion periods this decreased to 0.7%/hr. After 20 hrs of demecolcine, only 27% mitotic figures are observed. A rate of accumulation of blocked metaphases less than the true rate of cell production may arise (*a*) from inhibition of entry of cells into mitosis by the administered agent; (*b*) from incomplete blockade due to an insufficient dose of blocking agent; or (*c*) from disintegration and loss of the blocked metaphases due to an excessive dose of blocking agent (18). Since our demecolcine metaphase blockade is complete and cells blocked in metaphase do not appear to be lost during the time in question, it remains likely that cycling cells are being delayed and damaged during interphase growth in a manner that cannot be determined from the present experiments. It is of interest that studies utilizing murine bone marrow also suggest demecolcine-induced interphase cell loss (19). Tubulin is synthesized during the latter S and G_2 periods (4), and cells may show unusual sensitivity to demecolcine during this period of their growth.

There is a possibility that the fall in LI following the initial 4-hr interval (fig 5) results from demecolcine inhibition of DNA synthesis and death of labeled cells from unbalanced growth. This appears less likely since 4 hrs of demecolcine infusion at 6 μg/hr does not inhibit tumor cell grain count following ^{3}H-thymidine administration. A much higher infusion rate (18 μg/hr) clearly inhibits both grain count and the number of labeled cells (ie, DNA synthesis). This higher rate results in a net decrease in MI in comparison to infusions at 6 μg/hr (fig 8); the decrease may reflect arrest of cycling cells in S phase although there is the possible contribution of additional damage to premitotic or mitotic cells.

Our results are not entirely consistent with certain tissue culture studies that utilize Chinese hamster cells (20) and S_3 HeLa cells (21). In both preparations the mean generation time is determined directly by cinematography, and the effect of demecolcine addition on cell growth is subsequently estimated. Demecolcine does not arrest cells already in mitosis (21), and at the demecolcine concentrations used, the Chinese hamster cells accumulate in mitosis for 10–15 hrs and then escape from mitotic arrest while demecolcine is still present in the medium. In both cell preparations, mitoses accumulate at the rate anticipated from known generation time values during the initial 7–10 hrs following demecolcine addition, but the Chinese hamster cells show an accumulation rate that is lower than expected at later times. Another series of experiments (22) studied the rate of accumulation of metaphases in the rat crypt of Lieberkühn following ip injection of demecolcine or vinblastine, and compared these values to the rate of entry of these cells into mitosis as determined by a ^{3}H-thymidine autoradiographic method. The rate of cell production during the initial 2.5 hrs following demecolcine or vinblastine addition is approximately the same as that determined by the autoradiographic method, and it is again suggested that these agents, at suitable doses, do not affect the rate of entry of cells into mitosis. However, there are other reports that the vinca alkaloids colchicine and demecolcine have distinct effects on interphase cell growth (16,17,19,20,23–25), and the present experiments demonstrate a lower than expected rate of accumulation of mitotic cells during demecolcine administration, which we ascribe to injury and loss of nonmitotic cells. The type of demecolcine effect, its onset of action, and its duration of action probably depend on the demecolcine concentration at the tissue level and the tissue studied.

Since HeLa cells and Chinese hamster cells in tissue culture are capable of completing mitosis following reversal of colchicine or demecolcine metaphase blockade (5,11,20), they clearly retain the capacity to reorganize a functional bipolar spindle. Chinese hamster cells are also reported to undergo a second burst of mitotic activity 10 hrs after release from a 2-hr demecolcine blockade (11), indicating not only reversal of the blockade but also transient synchrony of the released cells. In vivo experiments have demonstrated mitotic accumulation in the small intestine of the rat following a single injection of demecolcine (15); since there was no evidence of tissue damage following the demecolcine blockade, it was thought that these cells are also capable of reforming a functional spindle and completing mitosis. However, synchronous recycling could not be demonstrated. In the present experiments there is partial recycling of tumor cells following release from demecolcine (fig 9). The 4-hr mitotic peak is ten times control mitotic rates and the 20-hr peak is twice control rates. The 20-hr value most likely represents recycled, synchronized cells. This further supports the contention that these cells may recover their ability to complete mitosis and offers an in vivo demonstration of partial synchronization of this tumor following demecolcine administration.

There is a strong similarity between the action of demecolcine and that of several other cancer chemotherapeutic agents. Vincristine, which is known to rapidly bind to tubulin with high affinity (26), has at least two distinct actions. Synchronized cells have a delayed entry into mitosis if continually exposed to vincristine (23,27). In HeLa cells, the duration of this delay is similar irrespective of whether the vincristine is added during G_1 or G_2, indicating that vincristine prolongs the interphase during the G_2 period (23). A similar interphase effect of vincristine or vinblastine, also reflected by a block in G_2, has been demonstrated using several other tumor cell lines (24,27). In addition, HeLa cells exposed to vincristine only during S phase do not exhibit a delay in passage through G_2 but are arrested during the following mitosis (23). Two separate effects of the vinca alkaloids are therefore apparent, one manifest in mitosis and the other in interphase cells. Since cells exposed to vincristine or vinblastine at concentrations that will greatly prolong the duration of metaphase (27) or induce spindle dissolution (25) may still retain the ability to subsequently form colonies, it is not necessary that metaphase arrest or spindle damage correlates with the primary lethal action of this drug. Podophyllotoxin and epipodophyllotoxin may exhibit the separation of these types of action in two related compounds. Podophyllotoxin binds to tubulin, inhibits its polymerization into microtubules, and ar-

rests cell division in mitosis (28). The epipodophyllotoxins, however, do not inhibit microtubule polymerization at equivalent concentrations (28) but reduce the mitotic index in several cell lines secondary to a premitotic arrest, with accumulation of cells in the G_2 cycle phase (29,30). Colchicine, demecolcine, the vinca alkaloids, and the podophyllotoxins appear to share to a greater or lesser extent the capacity to arrest mitosis secondary to an interaction with tubulin, and in an independent action, to produce a delay in interphase growth, with associated cell damage.

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Pulmonary Toxicity Studies of Bleomycin and Talisomycin[1,2]

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SUMMARY

The comparative pulmonary toxicity induced by bleomycin and talisomycin (former trivial name: tallysomycin A) was evaluated by measuring lung hydroxyproline content. Based on published subacute toxicity data, in which talisomycin was found to be four times as toxic as bleomycin, the following doses of each drug were given sc to BDF_1 male mice twice weekly for 4 weeks: 2.5, 5, and 10 mg/kg of bleomycin and 0.625, 1.25, 2.5, and 3.75 mg/kg of talisomycin. Equivalent dose-related effects in body weight were produced by bleomycin and talisomycin at a respective dose ratio of 4:1. Similarly, equivalent dose-dependent increases in lung hydroxyproline content, an index of collagen, were produced by bleomycin and talisomycin. Neither drug caused deaths at the doses used.

[Cancer Treat Rep 65:291–297, 1981]

Bleomycin, an antitumor antibiotic isolated from *Streptomyces verticillus* by Umezawa et al (1), is used extensively in treating human cancer. A complication of bleomycin treatment is a dose-related pulmonary toxicity which histopathologic studies have shown to be a drug-induced pulmonary fibrosis in man and in experimental animals (2,3).

Tallysomycin is a complex of new glycopeptide antibiotics isolated and identified by Kawaguchi et al in 1977 (4). It is active against Walker 256 carcinosarcoma in ascitic form with a minimum effective dose of about one quarter that of bleomycin (5). Talisomycin is 3.1 to 4.1 times more toxic than bleomycin in mice based on the single-dose ip LD50 and the multiple-dose (daily for 9 days) ip LD50, respectively (5). Talisomycin has the spermidine side chain associated with bleomycin A_5 with a β-lysine molecule inserted between the spermidine side chain and the nucleus (6). The nucleus of the talisomycin molecule also contains a unique amino sugar, talose.

Sikic et al (7) have demonstrated that bleomycin-induced increases in lung hydroxyproline (HDP) in mice can be correlated with histopathologic changes indicative of pulmonary fibrosis. The purpose of our study was to compare bleomycin and talisomycin at a 4:1 dose ratio which was established in acute toxicity studies (5). The drug-induced pulmonary toxicity was evaluated by measuring lung HDP content.

METHODS

Male BDF_1 mice weighing 20–25 g were provided with Wayne Breeder Blox and water ad libitum. Bleomycin was administered sc at doses of 2.5–10 mg/kg twice weekly for 4 weeks while talisomycin was administered to a separate group of mice in the same manner at doses of 0.625–3.75 mg/kg. The dose selection was based on the earlier work of Sikic et al (7) with bleomycin and on acute toxicity studies (5). The present experiments contain the results of several separate studies using different doses of bleomycin and talisomycin, including one study with bleomycin alone. The experimental design of these experiments is shown in table 1.

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TABLE 1.—Experimental design

Experiment No.	Drugs	Dose (mg/kg)	No. of groups*	No. of mice/ group†	Time (wks)‡
1	Control	—	2	70–80	4,6,8,10,12,14,19
	Bleomycin	10			
2	Control	—	5	45–55	6,8,10,12,14
	Bleomycin	10			
	Talisomycin	3.75,2.5,1.25			
3	Control	—	5	55	5,6,8,10,12
	Bleomycin	5,2.5			
	Talisomycin	1.25,0.625			

*Mice used in the individual experiments were randomly distributed among the groups.

†No. of mice in each of the groups at the beginning of the experiment; 10 mice were randomly selected from each group to be killed at each time interval. At the last date mice were killed, there sometimes were < 10 mice left in the group.

‡Wks after beginning of experiment, at which times mice were killed.

Ten mice from each group were killed starting at the termination of the 4-week dosing period and every other week thereafter to the 12th or the 14th week, depending on the experiment. In one instance, a group of mice was killed at the 19th week. The lungs were dissected from their blood vessels and bronchi and hydrolyzed at 110°C for 16–18 hours in 6 N HCl. The mixture was neutralized with NaOH, clarified, and filtered. An aliquot of the diluted filtrate was assayed for HDP using the method of Woessner (8). A tissue blank was used consisting of hydrolysate to which perchloric acid was added before chloramine-T. Duplicate samples including a tissue blank from each lung hydrolysate, and also standard HDP samples, were analyzed for HDP content on two separate occasions. The results were averaged before analyzing the data.

To compare data obtained in multiple dosing ex-

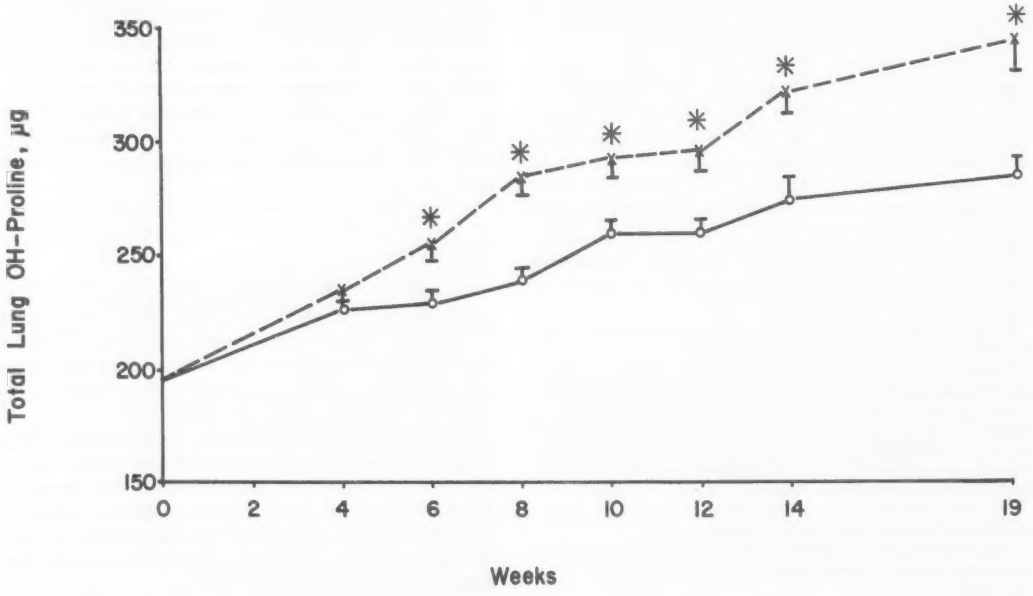

FIGURE 1.—HDP content (mean ± SE) of lungs after bleomycin treatment. -o- = control; and --x-- = bleomycin (10 mg/kg). * = significantly different from controls, $P < 0.01$.

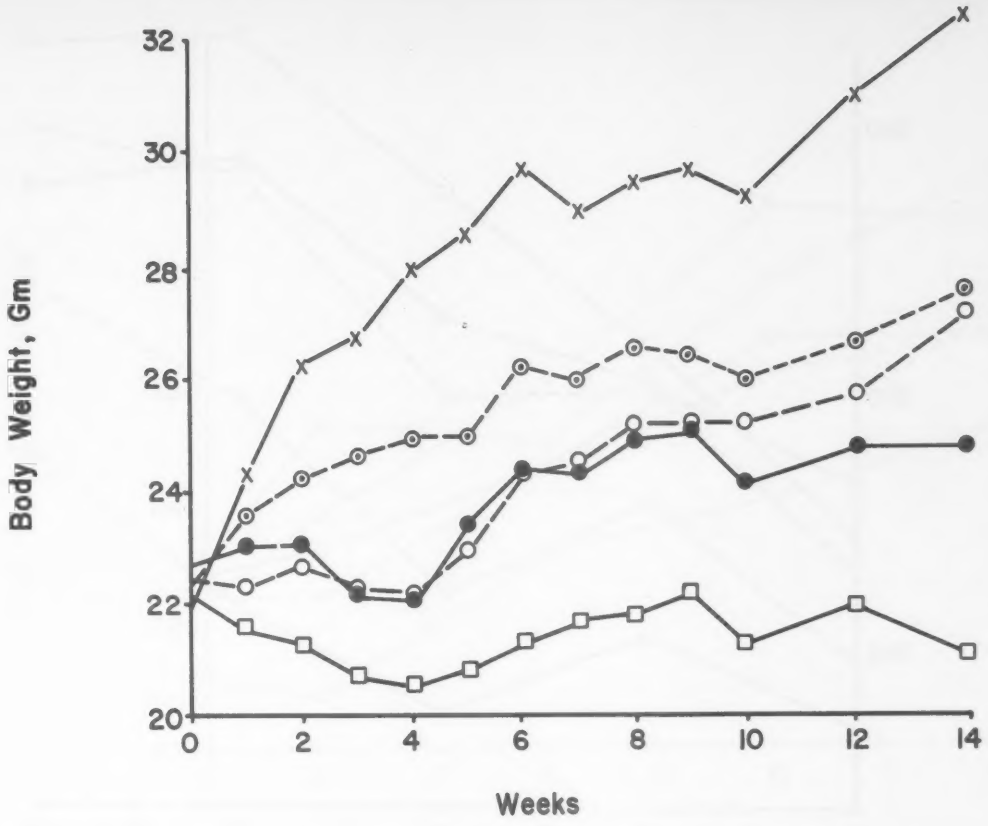

FIGURE 2.—Mean weight of mice during and after bleomycin and talisomycin treatment. -x- = control; -□- = talisomycin (3.75 mg/kg); --○-- = bleomycin (10 mg/kg); -●- = talisomycin (2.5 mg/kg); and --⊙-- = talisomycin (1.25 mg/kg).

periments, analysis of variance was done using the Student-Newman-Keuls procedure. Where two groups were compared, Student's t test was used. Analysis of data in the time course was done by two-way analysis of variance and comparison of regression lines was done by analysis of covariance (9). All statistical analyses were tested for significance at the $P = 0.05$ level.

RESULTS

Mice receiving bleomycin showed a significant increase in lung HDP content from the sixth week (2 weeks after completion of dosing) to the 19th week (fig 1). Since the slight increase in lung HDP content found at the fourth week was not significant, we did not sample HDP in later experiments until the fifth or sixth week.

During the 4 weeks of dosing, only the mice receiving the largest dose of talisomycin (3.75 mg/kg) lost weight (fig 2). Following dosing, all treatment groups maintained or began to gain weight, although none of the treatment groups attained the weight of the control group. It should be noted that after 6 weeks, the number of mice in each group decreased because mice were killed for HDP determination. Bleomycin at a dose of 10 mg/kg and talisomycin at a dose of 2.5 mg/kg produced a similar pattern of weight loss and gain, indicating that a 4:1 dose ratio of bleomycin to talisomycin was appropriate for subsequent experiments.

Figure 3 indicates the effect of bleomycin and

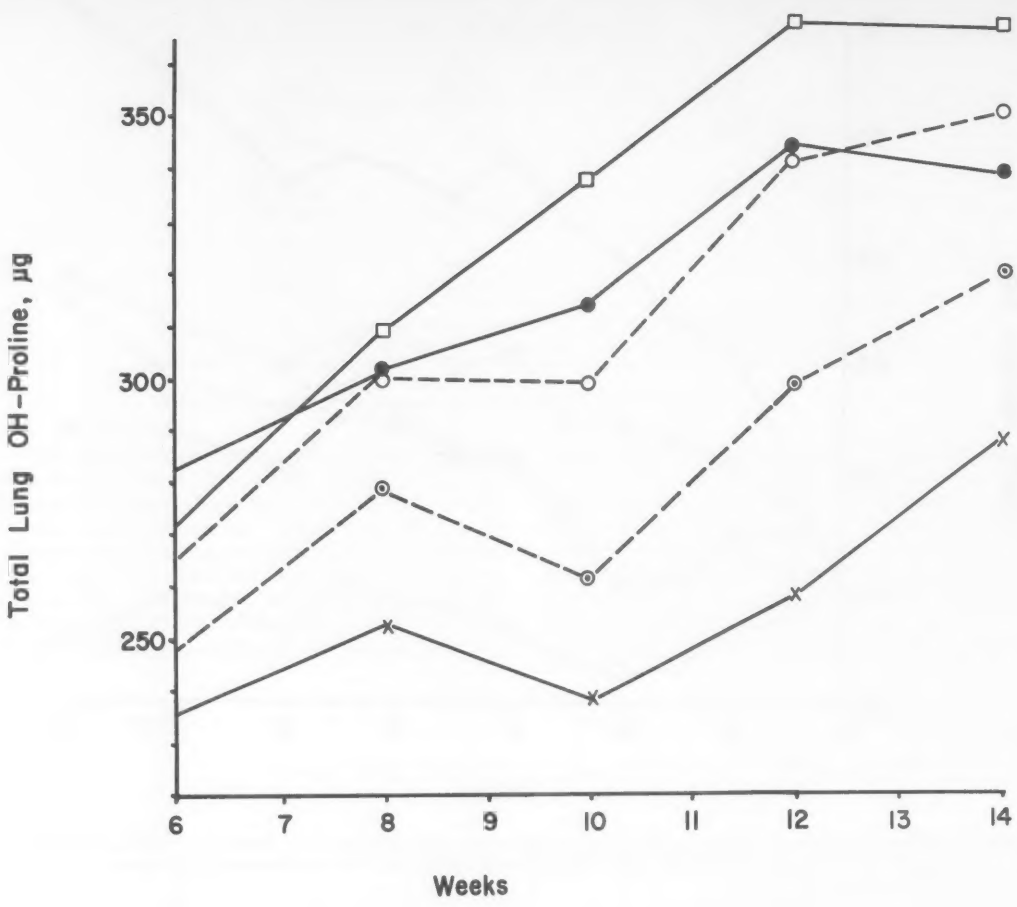

FIGURE 3.—HDP content of lungs after bleomycin or talisomycin treatment. -x- = control; -□- = talisomycin (3.75 mg/kg); --○-- = bleomycin (10 mg/kg); -●- = talisomycin (2.5 mg/kg); and --⊙-- = talisomycin (1.25 mg/kg). Relationship of group means across time: x □ ○ ● ⊙ (groups not connected here by a solid line are significantly different, $P = 0.05$).

talisomycin on lung HDP in the same group of mice mentioned in figure 2. Talisomycin increased lung HDP in a dose-related manner at each time point and over the time course of the experiment. All talisomycin groups were significantly different from each other and also from the control group, as shown by analysis of variance. Bleomycin at a dose of 10 mg/kg and talisomycin at a dose of 2.5 mg/kg produced equivalent increases in lung HDP. During the sixth to the 12th week of the experiment, the control mice showed an increase of approximately 9% in HDP while talisomycin at the highest dose (3.75 mg/kg) increased lung HDP by approximately 35%. Bleomycin at a dose of 10 mg/kg and talisomycin at a dose of 2.5 mg/kg produced parallel increases in lung HDP of approximately 26%. The lowest dose of talisomycin increased lung HDP by approximately 20%. We chose the 12th rather than the 14th week for comparing HDP means because results obtained for the 14th week were computed on less than ten mice per group.

Figure 4 illustrates an experiment similar to that shown in figure 3, except that lower doses of bleomycin and talisomycin were used. Analysis of co-

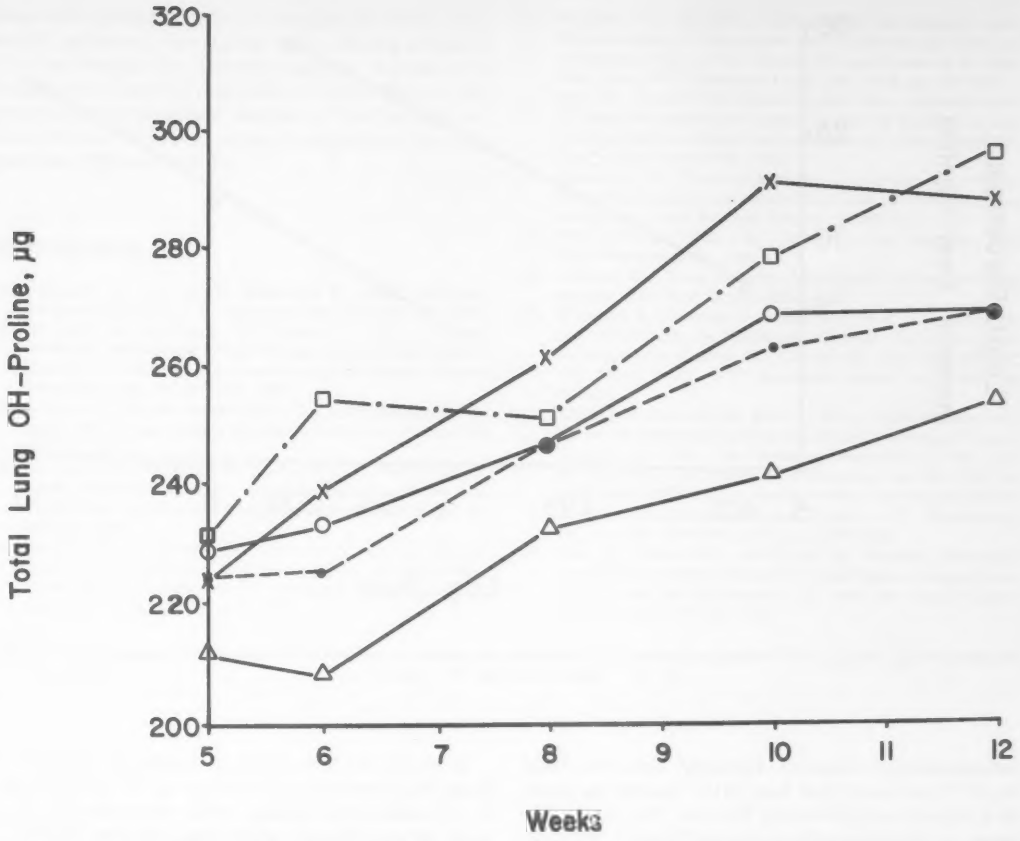

FIGURE 4.—HDP content of lungs after bleomycin or talisomycin low dose treatment. -Δ- = control; -x- = bleomycin (5 mg/kg); ·-□-· = talisomycin (1.25 mg/kg); -o- = bleomycin (2.5 mg/kg); and --●-- = talisomycin (0.625 mg/kg). Relationship of group means across time: Δ x □ o ● (groups not connected here by a solid line are significantly different, $P = 0.05$).

variances and regression lines indicated that bleomycin at doses of 5 and 2.5 mg/kg produced significantly different amounts of lung HDP. The same was true for talisomycin at doses of 1.25 and 0.625 mg/kg. Values for all treatment groups were significantly different from the control value. Thus, bleomycin and talisomycin produced statistically similar increases in lung HDP at two dose levels, with a dose ratio of 4:1 between drugs, and also showed a parallel dose response. Similarly, these same doses produced equivalent body weight changes (data not shown).

If the HDP results obtained in various experiments were expressed as a percent of the control value, they could be plotted on a logit-log dose graph. This type of graph is useful in comparing data obtained from various experiments and also produces a straight-line plot from a sigmoidal plot. Figure 5 is such a graph for the 12th week of the experiment. A pair of parallel lines was obtained indicating that a 4:1 dose ratio of bleomycin and talisomycin produced equivalent dose-related increases in mouse lung HDP. Similar plots and results (not shown) were obtained for other time periods.

DISCUSSION

It is time-consuming and difficult to quantitate bleomycin-induced pulmonary toxicity in mice using

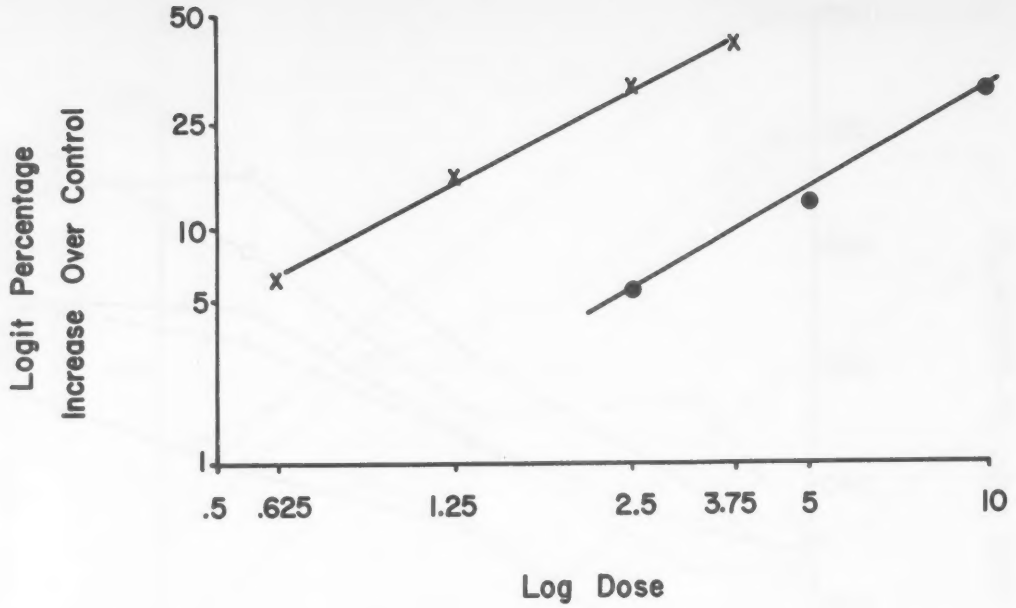

FIGURE 5.—Logit of lung HDP content expressed as % increase over control vs log dose of bleomycin and talisomycin at the 12th wk. -x- = talisomycin; and -●- = bleomycin.

histopathologic evaluation. Recently, however, Sikic et al (7) proposed that lung HDP content be used as a measure of pulmonary fibrosis. This method is based on the earlier observation of Crystal (10) that collagen is the most abundant of the structural components of lung tissue, and it could be quantitated by measuring the increase of lung HDP. Matsuda et al (11) were able to show that increasing doses of bleomycin produced a dose-related increase in lung lesions as shown by histopathologic evaluation. We (12), as well as Sikic et al (7), have shown that mice receiving bleomycin developed measurable pulmonary toxicity as demonstrated by histopathologic evaluation. Furthermore, using doses of bleomycin comparable to those used by Matsuda et al (11), the current study has also indicated that lung HDP content increased in a dose-related manner. Based on these observations, it appears that lung HDP measurements correlate with pulmonary toxicity induced by bleomycin in mice. Since we have also shown in this study that talisomycin parallels bleomycin in drug-induced lung HDP changes, it is likely that talisomycin also produces dose-related lung lesions, indicative of toxicity.

It should be noted that a significant increase in lung HDP content is first seen by the sixth week, ie, 2 weeks after dosing, while maximum development of morphologic alterations requires waiting 1 month past a 6-week treatment period (6) or 5 weeks past 10 daily doses (11). This suggests that the HDP assay is a simpler method and possibly an early indication for quantitating bleomycin-induced pulmonary toxicity.

Talisomycin has been shown to be active against several transplantable tumor systems with an effective dose range that is 2.5–11.5 times lower than that of bleomycin (6). Talisomycin was also found to be at least four times more lethally toxic for mice than bleomycin when given as a single ip dose or by a multiple-dose schedule (daily for 9 days) (5). Recently, Sikic et al (13) have indicated that talisomycin was two to three times as potent as bleomycin in producing pulmonary toxicity. Similarly, utilizing nonlethal doses of bleomycin and talisomycin (2.5–10 and 0.625–3.75 mg/kg, respectively), we have shown that a 4:1 dose ratio of bleomycin to talisomycin produced equivalent pulmonary toxicity in mice as measured by lung HDP. These same

doses also produced similar changes in mouse body weight patterns. Our results with a dosing schedule of twice weekly for 4 weeks support the use of a threefold to fourfold dose ratio of bleomycin to talisomycin in comparison studies of the biologic effects in mice. However, other dosing schedules may produce different results.

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Evaluation of Madison 109 Lung Carcinoma as a Model for Screening Antitumor Drugs[1]

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SUMMARY

The Madison 109 lung carcinoma (M109) was evaluated as a model for the screening of antitumor agents. Thirty-five drugs with established antitumor activity were assayed in mice implanted ip or sc with M109. Depending on the mode of tumor implantation, drugs representing those affecting nucleic acids (through binding, intercalating, or inducing single-strand breaks), various alkylating agents, mitotic inhibitors, antimetabolites, and immunomodulators were able either to inhibit the growth of sc M109 or to extend the lifespan of mice given M109 ip. The ip implanted tumor was, for example, markedly affected (median survival time of treated/control mice, × 100: ≥ 200% with occasional cures) by doxorubicin, mitomycin C, 10-hydroxy camptothecin, and dihydroxyanthraquinone. The sc implanted tumor, however, was markedly affected (treated − control of ≥ 12 days with regard to median time to grow 1-g tumors) by bleomycin and an analog, talisomycin, and by 6-thioguanine. The M109 was responsive to many different classes of clinically active agents and can serve as a useful tool in the screening of drugs with such potential. It may be particularly useful in screening analogs of camptothecins, nitrosoureas, bleomycins, and mitomycins as well as for evaluating anthraquinones, anthracyclines, mitotic inhibitors, antimetabolites, and immunomodulators.

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Primary lung carcinomas are the most frequent cause of cancer deaths in American men and the third most frequent cause in American women (1). Mortality rates continue to rise, and lung cancers, in general, have not responded well to available chemotherapy. The search for better animal models of human neoplastic diseases involves the establishment of positive correlations in drug sensitivity between the animal tumors and those tumors occurring clinically. Although there is no a priori reason to suppose that an animal lung tumor model will predict more reliably for the treatment of human lung tumors (or any other histologic type of human tumor) than some other in vivo tumor model, both the lack of attention paid to animal models of lung cancer (2) and the impact of this disease clinically makes such tumor models a reasonable area for experimental chemotherapy investigations.

In an attempt to provide an additional tool capable of selecting new agents for the treatment of human solid malignancies, several investigators have evaluated the Madison 109 lung carcinoma (M109) and have published their results characterizing the tumor's sensitivity to various agents (3–7). Marks et al (7) concluded that M109 had characteristics which warranted its further evaluation as an experimental model.

Prompted by these reports, M109 was added to the tumor panel at Bristol Laboratories and has been evaluated for > 1 year. The present report describes our experience with M109 and differs from previous investigations in that the tumor was im-

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planted ip and sc rather than im. Using both percent increase in lifespan (ILS) and delay in tumor growth as endpoints, the activity ratings of several drugs were found to differ substantially depending on the site of tumor implantation, and such differences in activity also obtained when the present data were compared with the results of previously published studies using im implanted M109 (3–7).

MATERIALS AND METHODS

Animals and Tumors

BALB/c and (BALB/c × DBA/2)F_1 (CDF_1) mice were used. The M109 was maintained in serial sc passage by transplantation of tumor brei in BALB/c mice every 2 weeks. For chemotherapy and surgical adjuvant chemotherapy trials, M109 was implanted ip or sc (in the flank, midway between the inguinal and axillary regions) into CDF_1 hosts.

Tumor breis were prepared by removing the sc growing tumors from the passage mice, dissecting away any obviously necrotic areas, weighing the tumors, mincing them with scissors and scalpel, suspending the minced fragments in cold Eagle's basal medium with Hanks' salts and L-glutamine (Grand Island Biological Co, NY), adding penicillin and streptomycin, homogenizing the suspended tumor fragments (using a Potter-Elvehjem-type apparatus) for approximately 20 secs (two bursts of 10 secs each), and suspending the homogenate to the desired concentration on a weight per volume basis.

From initial pilot studies (not reported), it was determined that implants made on the basis of percent tumor breis gave reproducible mortality and tumor growth rates that were as good as or better than those of implants made on the basis of counted numbers of cells (total cells or cells excluding trypan blue). With but few exceptions, the drug screening data to be presented have been obtained using CDF_1 mice implanted ip with 0.5 ml of a 2% brei or implanted sc with 0.1 ml of a 2% brei. Included in each experiment was a control group of mice that received the tumor implant inocula but no drug therapy, and usually an additional control group that received implants representing 1/10 of the dose of tumor cells given other groups of mice (referred to as the "1/10" control group). Each test and control group consisted of eight mice, but occasionally consisted of seven.

Drug Treatment

Mice were implanted with M109 and drug therapy was begun on Day 1 after implant. Treatment regimens consisted of either Day 1 only, Days 1 and 4, or Days 1–4, depending on the drug being studied. All drugs were evaluated at a minimum of two doses in at least one experiment at levels estimated (based on historical experience in other tumor models) to be within the effective dose range and probably approximating the maximum tolerated dose for each treatment regimen used. Typically, but not always, the dose levels chosen included those causing some drug-induced lethality so as to ensure that a maximum tolerated level had been reached. All injections were given ip in 0.5-ml volumes and were delivered on a milligram per kilogram of body weight basis using the initial (ie, Day-1) average body weight of the group of mice to be injected. All mice initially weighed between 18 and 23 g, and even then a mouse was not used if its weight was very different from that of the other mice in the same cage.

Assessment of Drug Activity

For mice implanted ip with M109, drug effects were evaluated on the basis of median survival time (MST) and incidence of long-term survivors ("cures") on Day 60. The MST of drug-treated mice was divided by the MST of untreated (no vehicle) control mice and multiplied by 100 to derive a % treated (T)/control (C) value. This calculation was made for all drug-treated groups unless fewer than six of eight mice were alive on Day 10 after implant, in which case the treatment was considered to be too toxic and no calculation was made. A drug was presumed to be active if (*a*) the MST of a treated group was greater than the MST of the control mice receiving 1/10 the tumor inocula, *and* (*b*) a T/C of ≥ 125% was achieved. In the event that criterion (*b*) but not (*a*) was fulfilled, the drug was described as having borderline (+/–) activity.

Among mice implanted sc with M109, drug effects were determined by comparing tumor growth in treated versus untreated (no vehicle) control mice. Two types of endpoints were used as the basis for comparison: (*a*) the difference in median times for tumors to grow to 1 g (T – C) and (*b*) the percent difference in median tumor weights determined 17–22 days after implant (% T/C). Tumors were measured twice weekly beginning about 1 week after implant and estimates of tumor weight were obtained by applying the formula: Wt (mg) = $a \times b^2/2$, where a = length (mm) and b = width (mm) of the tumor. The choice of which particular day of measurement, during Days 17–22 after implant, would be used for determining the T/C ratios in a given experiment was based on which of the days

a T/C value closest to 50% was found when the median tumor weights of the two control groups (those mice receiving the 2% and 0.2% breis) were compared. Presumptive drug activity was based on (*a*) inhibiting tumor growth to a level comparable to that seen in the parallel group of control mice implanted with the 0.2% tumor brei (ie, "1/10" control group), *and* (*b*) achieving a T – C of ≥ 4 days *or* a T/C of ≤ 50%. If criterion (*b*) but not (*a*) was fulfilled, the drug was described as having borderline (+/–) activity.

MST was not used routinely to evaluate drug activity against sc tumors because it was found not to correlate well with the levels of tumor inocula used (which differed by only 1 $\log_{10}$).

Surgical Adjuvant Chemotherapy

Mice were implanted sc with 0.1 ml of a 2% M109 brei and were kept together until the days on which surgery was performed. On such a day (Day 10 after implant in the one experiment to be presented in detail) mice were selected whose tumors were within a predetermined size range, eg, 200–400 mg. Except for an untreated control group, surgical removal of the tumor was done by resection using scissors, the wound was closed with surgical Autoclips (Clay Adams, B-D Co, Parsippany, NJ) under anesthesia (pentobarbital), and the mice were then distributed into different cages (ten mice per cage) for possible additional therapies. One group, the surgery control mice, received no further treatment, but the other groups received different chemotherapies. Mice were held for at least 90 days after implant and were observed weekly for the return of a tumor at the surgical site. Dying mice were autopsied and examined for the overt appearance of metastatic tumor (occurring primarily in the lungs and liver).

Challenge of Cured Mice

A small group of mice cured of sc M109 by either surgery or surgical adjuvant chemotherapy were challenged 96 days following their initial tumor implantation with 0.1 ml of a 0.2% M109 brei inoculated sc in a site contralateral to the original implant site. They were followed until death and their tumors were measured twice weekly. Control mice (not age-matched) that had never been exposed to M109 were implanted with the identical tumor inoculum concomitantly.

RESULTS

M109 Implanted Ip

The narrow distribution of deaths among control mice in 35 experiments (fig 1) reflects the consistency of the mortality patterns developed following tumor implantation by the ip route at the inocula levels used. For example, by Day 18 (the approximate mean MST of mice receiving the 2% tumor brei), only 6.8% of the mice (19 of 279) receiving the 0.2% tumor brei had died. Similarly, by Day 22 (the approximate mean MST of mice implanted with the 0.2% tumor brei), only 3.2% of the mice (nine of 278) receiving the 2% brei had not yet died. In addition, only one of 278 mice and three of 279 mice survived 60 days following implantation of 2% and 0.2% tumor brei inocula, respectively.

Untreated tumor-bearing control mice implanted ip with 0.5 ml of a 2% M109 brei died in a reproducible fashion, with an average (± SD) MST of 18.1 (±1.4) days (35 experiments). Similarly, untreated tumor-bearing control mice receiving 0.5 ml of a 0.2% brei died, with an average (± SD) MST of 22.2 (±2.0) days (35 experiments). The average (± SD) difference in MST between the two control groups in each experiment was 4.1 (± 1.7) days (34 comparisons).

It was reasonable, therefore, to assume that among mice implanted with a 2% tumor brei, a reduction in tumor cell body burden of 1 $\log_{10}$ (or equivalent inhibition of tumor growth) had occurred when a drug treatment resulted in a prolongation of the MST of either 4.1 days (when compared to untreated mice receiving this implant), or to the MST of the parallel, untreated tumor-bearing control mice receiving the 0.2% brei. Dividing the MSTs of the two control groups, 22.2 and 18.1 days (difference of 4.1 days), and converting to a percent, we obtain a value of approximately 125%. Based on this calculation, 125% was used as the minimum % T/C value that would reflect a drug effect of acceptable activity (rating of "+"). A % T/C of > 150%, based on obtaining an MST 9 days longer than the average control MST of approximately 18 days, was considered indicative of a "++" rating for drug activity.

Thirty-five compounds with known antitumor activity were evaluated in mice bearing M109 implanted ip (table 1). Twenty-two of these 35 drugs (63%) were judged to have caused a biologically significant increase in MST in at least one experiment (most drugs were evaluated at least twice).

The most active drug evaluated against ip M109 was doxorubicin. It was found to produce at least

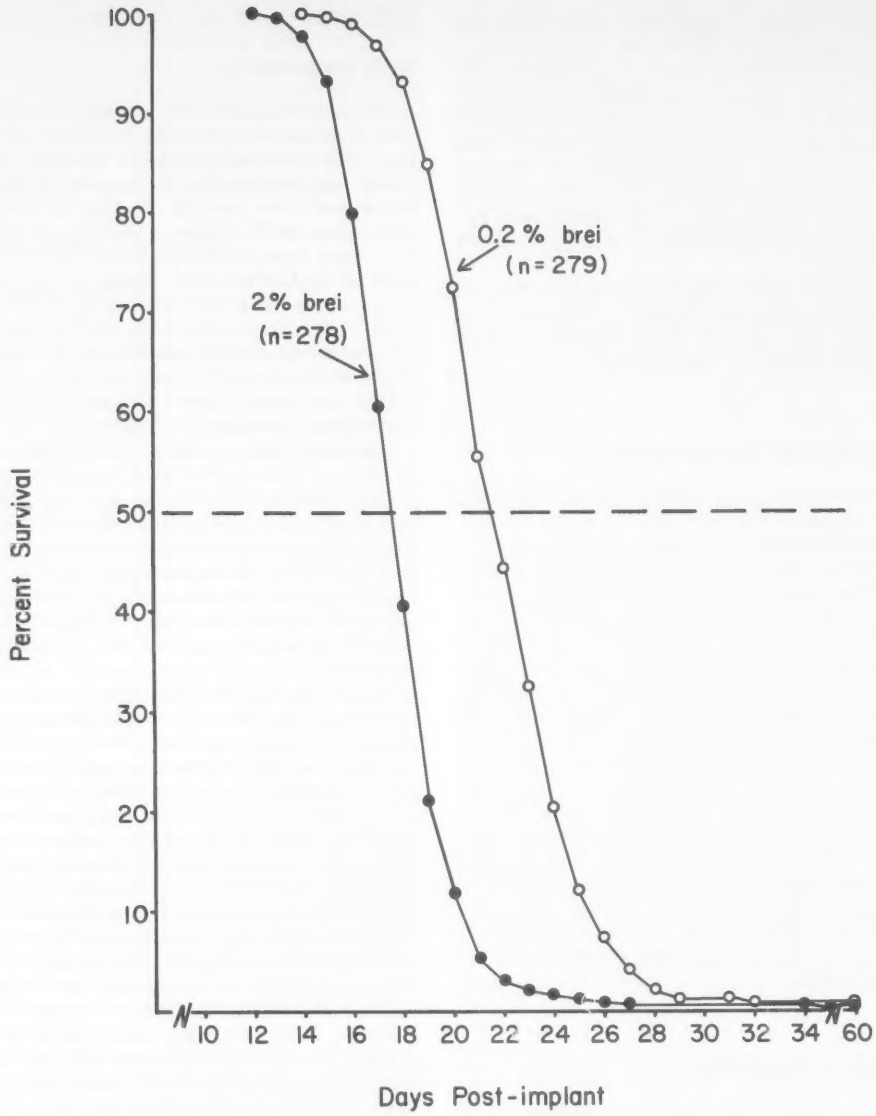

FIGURE 1.—Responses of untreated control CDF_1 mice implanted ip with 0.5 ml of either a 2% or a 0.2% M109 brei (35 experiments).

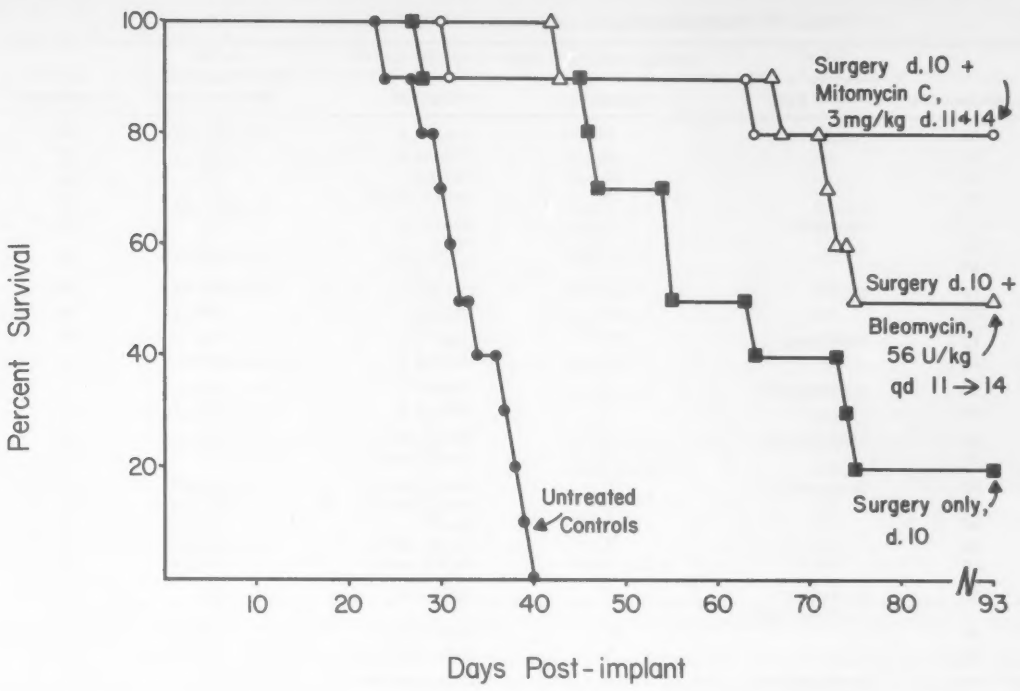

FIGURE 2.—Surgical adjuvant chemotherapy of CDF_1 mice bearing 200–400-mg sc M109. Treatment with mitomycin C or bleomycin alone (ie, no surgery), as early as Day 1 after implant (*see* table 2), was effective but noncurative at dose levels comparable to those used here.

one cure in each of the three experiments in which it was tested and consistently produced T/C values of almost 200% or more. Other anthracyclines, evaluated concomitantly with doxorubicin on two occasions (experiments 36 and 47), produced lesser effects ranging from inconsistent + activity (carminomycin), consistent + activity (marcellomycin), and + → + + activity (daunorubicin).

The only other drug to achieve a + + + + rating, ie, a ≥ 25% cure of the treated mice, was the 10-hydroxy analog of camptothecin. This drug initially caused a T/C of 176%, but when it was re-tested at higher doses it produced a T/C of 266%, including two of eight tumor-free survivors. Camptothecin itself was also active as was the 10-methoxy analog, but neither approached the level of 10-hydroxy camptothecin.

Based on a single experiment, dihydroxyanthraquinone (DHAQ) displayed marked activity in achieving a T/C of 244%, including one of eight mice cured. This result was good enough to award DHAQ a + + + rating.

The only other drug evaluated in this model to achieve a + + + rating was mitomycin C. In an initial experiment, mitomycin C therapy resulted in a T/C of 203%, including one of eight mice cured. In a repeat study, a single dose of mitomycin C given on Day 1 or multiple doses given on Days 1 and 4 (data not shown) yielded identical T/C values of 197%.

Other alkylating agents showed varied levels of antitumor activity. Chlorozotocin and BCNU both yielded T/C values consistent with a + + rating in two experiments. In contrast, methyl-CCNU (MeCCNU) produced a T/C of only 129% in one study (for a + rating) and was judged inactive in another experiment. Variable results were also found when evaluating cyclophosphamide and especially cisplatin. The former produced one therapeutic result consistent with a + rating, but was inactive in two other experiments. Cisplatin was judged to range from inactive to active (+ + rating). The results associated with cisplatin were the most variable of any of the 35 drugs evaluated in the ip

TABLE 1.—Optimal responses to chemotherapy of mice implanted ip with M109*

Experiment No.	Optimal treatment (ip)			% T/C (No. of cures/No. of mice in group)	Activity rating†
	Drug	mg/kg/dose	Schedule		
35	AMSA	2.5	Daily × 4	171	+ +
39		12	Daily × 4	163	+ +
43		15	Day 1	132	+
45		15	Days 1 and 4	161	+ +
17	Anguidine	0.8	Daily × 4	112	–
24		1.6	Daily × 4	95	–
42		0.8	Daily × 4	112	–
14	BCNU	40	Day 1	179	+ +
23		40	Day 1	173	+ +
12	Bleomycin	30	Daily × 4	114	–
13		30	Daily × 4	119	–
32	Camptothecin	2	Daily × 4	126	+
33		3	Daily × 4	141	+
24	Dactinomycin	0.2	Days 1 and 4	145	+
28		0.2	Days 1 and 4	149	+
20	Doxorubicin	5	Days 1 and 4	236(2/8)	+ + + +
26		5	Days 1 and 4	> 343(4/8)	+ + + +
36		12	Day 1	195(3/7)	+ + + +
36		4	Days 1 and 4	230(2/8)	+ + + +
46		4	Days 1 and 4	190(1/8)	+ + +
32	10-Hydroxy camptothecin	3	Daily × 4	176	+ +
33		12	Days 1 and 4	266(2/8)	+ + + +
33		6	Daily × 4	325(1/8)	+ + +
33	10-Methoxy camptothecin	5	Days 1 and 4	159	+ +
36	Carminomycin	0.5	Days 1 and 4	125	+
47		0.5	Days 1 and 4	113	–
44	Chlorozotocin	40	Day 1	162	+ +
18	Cisplatin	10	Day 1	129	±
28		6	Day 1	154	+ +
48		3	Daily × 4	123	–
23	Cyclophosphamide	300	Day 1	147	+
27		300	Day 1	79	–
31		300	Day 1	117	–
36	Daunorubicin	3	Day 1	160	+ +
47		3	Day 1	144	+
48	DHAQ	2	Daily × 4	244(1/8)	+ + +
37	DON	15	Days 1 and 4	141	+
41		15	Days 1 and 4	141	+
10	DTIC	125	Daily × 4	112	-
49		400	Day 1	100	-
37	5-FU	50	Days 1 and 4	118	–
41		75	Days 1 and 4	122	–
33	Homoharringtinone	2	Daily × 4	119	–
18	JM-5‡	20	Daily × 4	105	–
36	Marcellomycin	4	Days 1 and 4	128	+
47		3	Days 1 and 4	126	+
32	MeCCNU‡	20	Day 1	119	–
39		36	Day 1	129	+
37	6-Mercaptopurine	100	Days 1 and 4	174	+ +
41		150	Days 1 and 4	151	+ +

TABLE 1.—Optimal responses to chemotherapy of mice implanted ip with M109*—Continued

Experiment No.	Optimal treatment (ip)			% T/C (No. of cures/No. of mice in group)	Activity rating†
	Drug	mg/kg/dose	Schedule		
49	Methotrexate	30	Days 1 and 4	108	–
14	Mitolactol	150	Daily × 4	100	–
49		250	Daily × 4	100	–
28	Mitomycin C	2	Days 1 and 4	203(1/8)	+ + +
34		6	Day 1	197	+ +
28	Prednisolone	50	Daily × 4	103	–
49	Procarbazine	250	Daily × 4	105	–
21	Pyran	25	Daily × 4	154	+ +
22		25	Daily × 4	147	+
16	Sterigmatocystin	25	Daily × 4	95	–
16	Streptonigrin	0.2	Daily × 4	102	–
13	Talisomycin	12	Day 1	116	–
37	6-Thioguanine	30	Days 1 and 4	165	+ +
41		30	Days 1 and 4	132	+
21	VP-16-213	30	Days 1 and 4	146	+
26		50	Day 1	183	+ +
21	Vincristine	2	Day 1	141	+
26		1.5	Days 1 and 4	166	+ +

*Tumor implants consisted of 0.5 ml of a 2% brei in all experiments except No. 16 where 0.5 ml of a 0.2% brei was used. Additional control mice that received 1/10 of these tumor inocula (known as "1/10" control group) were included in all but experiment 32. Group sizes consisted of 7 or 8 mice (usually 8).

†+ + + + = > 200% T/C and at least 25% of the mice were tumor-free on Day 60 ("cures"); + + + = > 200% T/C but < 25% cures; + + = > 150% T/C; + = ≥ 125% T/C; ± = meets criteria for "+" rating but MST was not greater than that of "1/10" control group.

‡*See* text for definition of abbreviations.

M109 model; overall, the drug is considered to have a + rating. A platinum analog, *cis*-diamminehydroxymalonate platinum II (JM-5), was tested in parallel with cisplatin and was not found to be active.

The immunomodulator, pyran, was found to be active (+ → + +) against ip M109 and was included because of its reported effectiveness against im implanted M109 (3,5). Mice treated with pyran at 25 mg/kg/dose only, daily × 4, had T/C values of 147% and 154% in two experiments.

Another drug demonstrating good (+ +) activity was AMSA. Using multiple doses, AMSA produced T/C values of > 160% on three occasions.

An interesting spectrum of activities was associated with the testing of several antimetabolites. Two purine antagonists, 6-thioguanine and 6-mercaptopurine, achieved T/C values good enough for a + + rating, the latter drug on two occasions. The glutamine antagonist, DON, twice achieved a T/C of 141% for a + rating. Both 5-FU and methotrexate proved to be inactive.

Vincristine and VP-16-213 were also found to be active against ip M109 in two experiments. Maximum T/C values of 141%–166% and 146%–183% were obtained using vincristine and VP-16-213, respectively.

One other drug demonstrated modest but consistent activity in the ip M109 model. Dactinomycin caused increases in MST in two experiments (T/C of 145%–149%) and thus achieved a + rating.

Several drugs evaluated against M109 implanted ip were judged to be inactive, in addition to those already discussed. Among these were anguidine, bleomycin, mitolactol, DTIC, homoharringtinone, prednisolone, procarbazine, sterigmatocystin, streptonigrin, and a bleomycin analog, talisomycin (limited testing, ie, only 12 mg/kg, single injection).

M109 Implanted Sc

The median time to grow a tumor to 1 g varied slightly from experiment to experiment among mice implanted sc with 0.1 ml of either a 2% or 0.2% brei. Mice receiving the 2% brei had their tumors reach 1 g in median times of from 15 to 22 days (31 experiments), but the average (± SD) median time was

17.8 (±1.7) days reflecting fairly good reproducibility in growth rates. Similarly, mice receiving 0.2% brei implants grew their tumors to 1 g in median times ranging from 17.5 to 26.5 days (30 experiments); however, the average (± SD) median time was 21.3 (± 2.3) days.

With regard to the median time to reach 1-g tumors, reasonable consistency was observed in the time differential between the two control groups in each experiment. These differences ranged, in the extreme instances, from 1.0 to 8.5 days, but with an average (± SD) of 3.6 (± 1.8) days (28 comparisons). Using this average value, it was decided that a T – C of ≥ 4 days in a drug-treated group could reasonably be thought of as reflecting a biologically significant delay in tumor growth, and that drugs effecting this response be described as active ("+") *if* such a delay in growth was also greater than that observed in the parallel "$\frac{1}{10}$" inoculum control group.

Lifespan, as reflected by MST, was not routinely determined in either control or drug-treated mice. This was due to data collected in pilot studies in which MST was not found to correlate well with the different tumor inocula implanted sc. Similarly, of the 17 experiments reported here in which mice were held until reaching their MST, there were five occasions (29%) when control mice that received a 2% tumor brei inoculum lived as long or longer than control mice receiving only one tenth as great a tumor implant. Because a reduction in tumor body burden as great as 1 $\log_{10}$ would not necessarily be reflected by an increase in median lifespan, changes in lifespan were not used to assay for drug activity against sc tumors. For informational purposes, however, the mean MST (± SD) of mice receiving 0.1 ml of a 2% brei sc (17 experiments) was 37.2 (±2.0) days, with values ranging from 33 to 40.5 days. For mice receiving one tenth of this tumor inoculum, the mean MST (± SD) was 39.4 (± 4.0) days, with a range of 31–47 days (17 experiments). Finally, the average difference (± SD) between MSTs of the control groups in each experiment (17 comparisons) was 2.3 (± 3.0) days.

Thirty-five drugs were evaluated in mice implanted sc with M109 (table 2). Twenty-two of the 35 drugs (63%) were judged to have caused a biologically significant delay in tumor growth in at least one experiment. Nearly all drugs were evaluated at least twice.

Among the most active (+ + +) of the drugs evaluated against sc M109 were bleomycin and its analog, talisomycin. In an initial experiment (No. 12), bleomycin caused a T – C of 22.0 days. On Day 19 after implant, the median tumor weight in the bleomycin-treated group was only 4% of that of the untreated control group which had received the same initial tumor inoculum (ie, T/C: 4%). In a second experiment (No. 13), bleomycin yielded a T – C of 11 days and a T/C of 8%. It is of interest to note that these same regimens of bleomycin which proved so successful in treating sc M109 were without meaningful effects among identically and concomitantly treated mice bearing ip implanted M109 (cf experiments 12 and 13 in tables 1 and 2).

Included in experiment 13 were mice treated with talisomycin (4 mg/kg/dose, daily × 4 only), whose response was good enough to warrant a + + + rating. A more extensive evaluation of talisomycin was performed in experiment 34, where both it and bleomycin were evaluated concomitantly versus (only) sc M109. At its optimal dose, bleomycin caused a T – C of 15.0 days and a T/C of 15%. However, optimal treatment with talisomycin resulted in the failure of most primary tumors to reach 1 g before the mice died of metastatic disease (T – C of > 34.0 days). At one third to one half its optimal dose level, talisomycin still caused T – C values of ≥ 17.5 days and a T/C of 0–15% (data not shown).

In the experiments involving bleomycin and talisomycin, all mice implanted sc with M109 were held for comparison of their survival . a. Despite the ability of bleomycin to greatly suppress the growth of sc tumors, the MST T/C values of these successfully treated groups of mice were only 96%–113%. That is, inhibition of sc tumor growth was not reflected by meaningful ILSs. Optimal treatment with talisomycin resulted in only slightly better median ILSs as reflected by MST T/C values of 120%–140%.

Among alkylating agents, activities ranged from an unconfirmed + to a reproducible + +. DTIC produced the unconfirmed activity, and mitomycin C and cyclophosphamide were the most active, producing T – C values as great as 11.5 and 10.0 days, respectively. The only substantial inconsistency observed in testing drugs against this model involved cyclophosphamide. The drug was inactive in experiment 27, but achieved a + + rating in two other studies.

Among the nitrosourea alkylating agents, MeCCNU and BCNU (in separate experiments) demonstrated + → + + activity, and chlorozotocin, based on one study, was also active (+). On one occasion, MeCCNU definitely performed better against sc M109 than against ip M109 (cf experiment 32 in tables 1 and 2). On another occasion, MeCCNU produced one of the few ILSs (MST T/C of 124%) worthy of mention (from among the 17 experiments where lifespans were followed), despite only modest suppression of primary tumor growth.

Another alkylating agent, cisplatin, was also evaluated and repeatedly demonstrated weak activity (+) in this model. JM-5, a platinum analog, was not found to be active.

Among the anthracyclines evaluated, doxorubicin was clearly the superior agent based on results from four experiments, two of which included parallel testing of the other anthracyclines. The activity rating was generally + but reached ++ in the last experiment performed (No. 47) where both single- and multiple-dose drug administrations resulted in a T – C of 10.0 days, a T/C of 14%–15%, and an MST T/C of 126%. Daunorubicin and carminomycin each produced therapeutic results judged to reflect inactivity in one study and borderline activity in another. Marcellomycin was twice found to be inactive.

Concerning other agents known to bind to nucleic acids, both AMSA and DHAQ were found to yield delays in growth consistent with a + activity rating. On one occasion (experiment 39), an MST T/C of 125% was achieved using AMSA despite a T – C of only 4.0 days. Dactinomycin was twice found to be devoid of activity, although some transient inhibition of tumor growth was observed.

Among agents known to produce single-strand breaks in DNA, other than bleomycin and related analogs, streptonigrin was found to lack activity (based on one study) as was camptothecin. However, two analogs of camptothecin, the 10-hydroxy and the 10-methoxy derivatives, demonstrated activity (+) against sc M109.

Several antimetabolites were evaluated in the sc M109 model. The activity shown by 6-thioguanine (+++) was comparable to that seen using bleomycin. Moreover, the reproducible ILSs, reflected by MST T/C values of 149% and 159% in two experiments, were the best achieved by any drug evaluated by this parameter. Another purine antagonist, 6-mercaptopurine, twice caused delays in tumor growth consistent with a + activity rating, but also caused MST T/C values of 125% and 130% in the two studies. The glutamine antagonist DON was also quite effective against sc M109. Its activity was rated + → ++ on the basis of T – C values of 7.5–8.5 days with a T/C of 14%–28%. A pyrimidine analog, 5-FU, was found to be inactive in one experiment but weakly active (+) in another study. Methotrexate evaluated at two dose levels and on two different regimens was inactive in one experiment (a lethally toxic level was achieved).

VP-16-213 and vincristine were tested twice in parallel and each was found to be both comparable to one another and active (+).

Pyran, an immunopotentiator, was found to be effective against sc M109. Two different lots of pyran were evaluated at 25 mg/kg/dose, daily × 4 (experiments 18 and 20), or 25 and 50 mg/kg/dose, daily × 4 (experiment 40), and only one of them demonstrated activity (+). In the last experiment performed (No. 40), the optimal effects were found at the lower dose level, including an MST T/C of 127%.

Those drugs not yet mentioned that were evaluated in this model but were not found to be active include anguidine, homoharringtinone, prednisolone, and sterigmatocystin. Mitolactol and procarbazine were found to have borderline activity (±) in at least one experiment.

A few drugs were also evaluated using an iv route of administration (data not shown). The results of such investigations, involving pyran, anguidine, AMSA, and cisplatin, may be briefly summarized by stating that the therapeutic results obtained when using iv drug injections were not better than those obtained (in the same experiment, except for pyran) when using the ip route.

Surgical Adjuvant Chemotherapy of Sc M109

No mouse implanted sc with M109 had been cured using chemotherapy, and metastatic disease was known to be responsible for deaths even when primary tumors were greatly suppressed. Thus, the use of sc M109 as a model for surgical adjuvant chemotherapy seemed feasible.

The therapeutic results of a portion of one surgical adjuvant chemotherapy experiment are shown in figure 2 to illustrate the kind of data obtained. Untreated, tumor control mice died with an MST of 33.0 days. The other mice selected on Day 10 after implant for having sc tumors of between 200 and 400 mg received various treatments. Those whose treatment consisted of only surgical removal of tumors (on Day 10) had an MST of 59.5 days or a T/C of 180% compared to untreated control mice. Two of the ten mice treated in this manner survived to Day 93 and were considered cured. Of the mice that died, four had no primary site regrowth of their tumors and two others had only small (primary site) tumors. Metastatic disease, primarily to the lungs and liver, was apparent at death among those dying mice available for autopsy (some mice were cannibalized). Thus, metastatic spread of M109 had occurred in at least 40% of the mice by the time their primary tumors had been removed 10 days after implant.

When, in addition to surgery, mice received chemotherapy in the form of either mitomycin C or

TABLE 2.—Optimal responses to chemotherapy of mice implanted sc with M109*

Experiment No.	Optimal treatment (ip)			MST (% T/C)	T – C (Days to 1 g)	Tumor weight (% T/C)	Activity rating†
	Drug	mg/kg/dose	Schedule				
35	AMSA	8	Daily × 4	117	5.5	33	+
39		12	Daily × 4	125	4.0	52	+
45		15	Days 1 and 4	110	3.5	34	±
17	Anguidine	1.6	Daily × 4	—	(–2.0)	83	–
42		1.6	Daily × 4	—	(–1.5)	124	–
14	BCNU	25	Days 1 and 4	105	9.0	22	+ +
23		40	Day 1	—	7.0	37	+
12	Bleomycin	30	Daily × 4	96	22.0	4	+ + +
13		20	Daily × 4	113	11.0	8	+ +
34		36	Daily × 4	113	15.0	15	+ +
32	Camptothecin	3	Days 1 and 4	—	3.0	59	–
33		5	Daily × 4	—	2.5	66	–
32	10-Hydroxy camptothecin	6	Days 1 and 4	—	6.0	35	+
33		6	Daily × 4	—	3.5	34	+
33	10-Methoxy camptothecin	5	Days 1 and 4	—	5.5	19	+
36	Carminomycin	0.8	Day 1	84	2.5	87	–
47		0.5	Days 1 and 4	88	6.5	67	±
44	Chlorozotocin	40	Day 1	101	7.5	46	+
18	Cisplatin	10	Day 1	—	5.0	44	+
22		3	Daily × 4	—	5.0	47	+
48		10	Day 1	99	4.5	50	+
23	Cyclophosphamide	300	Day 1	—	9.5	14	+ +
27		200	Day 1	—	(–2.5)	121	–
31		300	Day 1	—	10.0	7	+ +
20	Dactinomycin	0.3	Days 1 and 4	—	2.0	51	–
28		0.3	Days 1 and 4	—	2.0	75	–
36	Daunorubicin	2	Days 1 and 4	96	0	130	–
47		3	Days 1 and 4	92	6.0	68	±
48	DHAQ	3	Days 1 and 4	101	6.0	41	+
37	DON	10	Days 1 and 4	121	8.5	14	+ +
41		90	Day 1	118	7.5	28	+
20	Doxorubicin	7.5	Days 1 and 4	—	4.0	36	+
26		12	Day 1	—	5.5	32	+
36		12	Day 1	110	9.0	34	+
47		12	Day 1	126	10.0	14	+ +
47		6	Days 1 and 4	126	10.0	15	+ +
10	DTIC	75	Daily × 4	119	4.5	47	+
49		400	Day 1	95	1.0	86	–
37	5-FU	50	Days 1 and 4	113	4.0	36	+
41		50	Days 1 and 4	107	0.5	82	–
33	Homoharringtinone	2	Daily × 4	—	2.5	60	–
18	JM-5‡	20	Daily × 4	—	3.0	56	–
36	Marcellomycin	4	Days 1 and 4	96	1.0	95	–
47		4	Days 1 and 4	105	2.0	82	–
32	MeCCNU‡	15	Days 1 and 4	—	10.0	17	+ +
39		40	Day 1	124	6.5	50	+
37	6-Mercaptopurine	100	Days 1 and 4	130	7.0	20	+
41		100	Days 1 and 4	125	8.0	31	+
49	Methotrexate	30	Days 1 and 4	101	2.0	92	–

TABLE 2.—Optimal responses to chemotherapy of mice implanted sc with M109*—Continued

Experiment No.	Optimal treatment (ip)			MST (% T/C)	T – C (Days to 1 g)	Tumor weight (% T/C)	Activity rating†
	Drug	mg/kg/dose	Schedule				
14	Mitolactol	400	Day 1	105	4.0	50	±
29		500	Day 1	85	0.5	98	–
28	Mitomycin C	4	Days 1 and 4	—	11.0	13	+ +
34		6	Day 1	109	9.5	26	+ +
46		3	Days 1 and 4	113	11.5	18	+ +
24	Prednisolone	25	Daily × 4	—	(–3.0)	130	–
28		30	Daily × 4	—	2.0	68	–
	Pyran						
18	Lot A	25	Daily × 4	—	5.5	47	+
20	Lot B	25	Daily × 4	—	1.0	88	–
40	Lot A	25	Daily × 4	127	8.5	41	+
49	Procarbazine	250	Daily × 4	114	4.0	61	±
16	Sterigmatocystin	50	Day 1	—	2.0	68	–
16	Streptonigrin	0.2	Daily × 4	—	2.5	57	–
13	Talisomycin	4	Daily × 4	120	14.0	9	+ + +
34		12	Daily × 4	140	∞§	0	+ + +
37	6-Thioguanine	30	Days 1 and 4	159	15.5	0	+ + +
41		40	Days 1 and 4	149	16.0	0	+ + +
21	VP-16-213	30	Days 1 and 4	—	4.0	49	+
26		50	Days 1 and 4	—	6.5	36	+
21	Vincristine	1	Days 1 and 4	—	7.0	34	+
26		1	Days 1 and 4	—	9.0	34	+

*Tumor implants consisted of 0.1 ml of a 2% brei except in experiment 16 where 0.1 ml of a 0.2% brei was used and in experiments 10 and 13 where 2–3 × 10^5 tumor cells/0.1 ml were used. Additional control mice that received 1/10 of these tumor inocula (known as "1/10" control group) were included in all but experiments 24, 32, and 42. Group sizes consisted of 7 or 8 mice (usually 8).

†+ + + + = T – C ≥ 12 days, T/C (tumor inhibition) ≤ 10%, *and* at least 25% of the mice tumor-free on Day 60 ("cures"); + + + = same as previous rating but < 25% cures; + + = T – C ≥ 8 days and T/C ≤ 25%; + = T – C ≥ 4 days *or* T/C ≤ 50%; ± = meeting criteria for "+" rating but tumor growth greater than present in "1/10" control group. MST % T/C (reflecting ILS) was not used in determining activity ratings.

‡*See* text for definition of abbreviations.

§Fewer than one half of the mice grew tumors of 1 g before death.

bleomycin, their survival rates increased to 80% and 50%, respectively. Recalling that early initiation of therapy with either drug alone produced no cures of mice bearing sc M109 (table 2) at dose levels comparable (note: 56 units/kg of bleomycin is equivalent to about 33 mg/kg) to those used in the surgical setting, the advantage of surgical adjuvant chemotherapy over either single treatment modality was evident.

Challenge of Mice Cured of Sc M109

The mice cured in the surgical adjuvant chemotherapy experiment shown in figure 2 were challenged on Day 96 (following their original tumor implant) with M109 implanted sc. Control mice that had never before been exposed to M109 and had received the identical "challenge" inoculum had an MST of 43.5 days. Mice cured by surgery plus mitomycin C showed no evidence of resisting the challenge inoculum (MST of 29.5 days). Those mice cured by surgery alone were too small a sample (two mice) to judge the influence of prior exposure on challenge. But the five mice cured using surgery plus bleomycin did demonstrate an increase in median lifespan (MST T/C of 120%) and a substantial delay in tumor growth reflected by a T – C of 17.5 days (T/C of 16%). Such suppression of tumor growth, despite the small sample size, suggests that an immunologic explanation may be involved in the resistance of these once-cured mice to challenge with M109.

DISCUSSION

The M109 has many characteristics desirable in an experimental tumor model to be used for screening drugs. These include, as also noted by Marks et al (7), the features of being transplantable, metastatic, recoverable from frozen storage, and yield-

ing reproducible growth and/or mortality rates (depending on the route of implantation). In the present study, M109 implanted ip induced reproducible mortality rates at two different inoculum levels separated by 1 $\log_{10}$. The extremely low "no-take" rate ($\leq$ 1%) found with either inoculum level obviates the need for skepticism associated with the achievement of drug-treated cures. Such mortality data compare favorably with the death patterns observed following ip implant of tumors such as B16 melanoma (B16) and Lewis lung carcinoma (LL) (personal observations). Similarly, reproducible rates of growth (to 1 g) of M109 implanted sc were demonstrated for inocula of approximately the same orders of magnitude as were used in the ip studies. In fact, the growth of sc M109 and the mortality data presented for ip M109 fall well within the limits of acceptability established for many of the tumor models used under the auspices of the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.[4] Although not quantified, the occurrence of "no-takes" among mice implanted sc with M109 was rare; however, sloughing of necrotic tumors did occur occasionally.

The establishment of a dose response for M109 implants over 1 $\log_{10}$ dilution was sufficient for the purpose of this study, ie, the screening of antitumor drugs. More accurate estimates of drug-induced tumor cell-kill (beyond 1 order of magnitude) would require the establishment of a reproducible dose-response relationship over several $\log_{10}$ dilutions. In pilot experiments (data not presented), 100% mortality was achieved following ip implant of 0.5 ml of a 0.002% brei. Marks et al (7) reported achieving 100% mortality among mice implanted im with 500 M109 cells and about 50% mortality in mice similarly implanted with 50 M109 cells.

Despite the finding that M109 is relatively resistant to cure by single-agent chemotherapy, the tumor was found to be sensitive to a number of drugs representing many different classes of antitumor compounds. Depending on the mode of tumor implantation, drugs representing those affecting nucleic acid (through binding, intercalating, and inducing strand breaks), various types of alkylating agents, mitotic inhibitors, antimetabolites, and immunomodulators were able either to inhibit the growth of sc M109 or to extend the lifespan of mice given M109 ip. For example, against ip M109, doxorubicin, BCNU, cisplatin, mitomycin C, cyclophosphamide, and the mitotic inhibitors, vincristine and VP-16-213, all demonstrated antitumor activity. Some of these same (or structurally related) drugs, doxorubicin (8–10), cisplatin (8,11), nitrosoureas (10,12), vincristine (10), cyclophosphamide (8, 10,12), and VP-16-213 (13), are used clinically (usually as part of combination therapies) against various forms of lung cancer. It would be presumptuous, based on the retrospective study performed, to consider M109 as a proven model for selecting drugs that will be clinically effective in treating lung cancers. The several drug activity correlations described should not be ignored; rather, the question of their significance should be held in abeyance pending additional demonstration of positive activity correlations, ideally from prospective drug leads.

Some differences in activity rating occurred when the panel of drugs was evaluated against sc M109. For example, doxorubicin, which consistently cured some mice implanted ip with M109, was only moderately active against sc M109. Conversely, bleomycin was devoid of any detectable antitumor activity versus ip M109 but was found to be among the most active of the agents evaluated in mice bearing sc M109. Also of interest were the similar activities of MeCCNU and BCNU against sc M109 but the superiority of BCNU against ip M109 (although both nitrosoureas were not compared in the same experiment). The 10-hydroxy analog of camptothecin, which is reported to have good clinical activity in China (14), was clearly superior to the other camptothecins when evaluated against ip M109 but was not outstanding when tested against sc M109.

Compared to other commonly used nonleukemia murine tumor models such as LL and B16, the most obvious differences in drug sensitivity involve some of the antimetabolites. Whereas M109 is quite sensitive to 6-mercaptopurine, 6-thioguanine, and DON, neither LL nor B16 is sensitive to these agents to the same degree, if at all [personal observation and (15)]. M109 shares with these tumors, however, utility as a model for surgical adjuvant chemotherapy and, additionally, immunochemotherapy.

Only a few other studies with M109 have been reported. Woodman and Gang (3) found a < 20% ILS among BALB/c mice bearing im implants of M109 when treated (beginning 1 day after implant) with cyclophosphamide, BCNU, methotrexate, ICRF-159, daunorubicin, vincristine, prednisone, or bleomycin. Pyran was found by these investigators to yield the best and most reproducible ILS (60%), and bacillus Calmette-Guérin and poly I:C also produced significant ILS values of 20%–30%.

In chemotherapy trials done at the Southern Re-

[4]Protocol Committee, Screening Section, Drug Evaluation Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Summary of the usual characteristics of selected murine models used under the auspices of the National Cancer Institute, Division of Cancer Treatment. Instruction 271C, 1979.

search Institute, Birmingham, AL, with M109 (D. Dykes, personal communication), the tumor responded well to cyclophosphamide, MeCCNU, and bleomycin, gave marginal responses to CCNU, BCNU, the 5′-palmitoyl ester of cytosine arabinoside, and *Corynebacterium parvum*, and was inhibited in its growth by 5-FU, doxorubicin, vincristine, and mitomycin C; negative or mixed responses were obtained with hexamethylmelamine, macromomycin B, VP-16-213, and cisplatin. These results were based primarily upon sc tumor growth comparisons (as in the present report) and not necessarily changes in lifespan.

The most extensive investigation made of the drug sensitivity profile and biologic characteristics of M109 was performed by Marks et al (7). They evaluated 82 compounds for the ability to increase the MST in mice implanted im with M109 cells. Only 9% of the compounds tested produced confirmed activity (ILS $\geq$ 25% in two or more experiments) and these included dactinomycin, doxorubicin, daunorubicin, procarbazine, and pyran. Unconfirmed activity (ILS $\geq$ 25% in one experiment) was obtained with 28% of the agents evaluated including bleomycin, DTIC, prednisone, and streptonigrin. About one half of the compounds tested were found to be inactive including cyclophosphamide, BCNU, MeCCNU, vincristine, melphalan, cytarabine, and mitomycin C.

The apparent discrepancies in drug activities reported in these studies and in the present series of experiments are partially resolved when one considers that they are the consequence of the many variables present including, but not limited to, site of tumor implantation, treatment regimens, use of antibiotics in preparing tumor breis, and endpoint analyses. For example, relatively slight delays in tumor growth were considered indicative of biologically significant antitumor activity in the present study, without regard to accompanying ILSs. However, in defense, only short courses of treatment (not exceeding 4 days) were used throughout the present series of experiments as opposed to the $\geq$ 9 days of therapy used in other drug evaluation studies. Marks et al (7), for example, used treatment regimens that extended > 17 days (for chlorodinitrobenzene compounds), 25 days (for dactinomycin), and even 33 days (for tilorone) in some instances. Similarly, the unpublished data emanating from the Southern Research Institute (D. Dykes, personal communication) included therapeutic results obtained using drug treatment regimens of up to 12 days' duration; however, the duration of treatment was taken into consideration when evaluating the magnitudes of the antitumor drug effects.

In conclusion, the M109 is responsive to many different types of clinically active antitumor agents and can serve as a useful tool in the screening of drugs with such potential. Based on the data presented, M109 might be particularly useful in screening analogs of nitrosoureas, camptothecins, bleomycins, and mitomycins, and for evaluating anthracyclines, anthraquinones, mitotic inhibitors, antimetabolites, and immunomodulators. Because of the discrepancies observed in activity when evaluating drugs in the sc and ip M109 models, we are unable to recommend one implant site as being preferable to the other for purposes of future screening; both models are still being used in our laboratory. Finally, M109 should not be thought of as a definitive screening model for drugs that fail, or are only weakly active, against P388 leukemia, since it was insensitive to streptonigrin, sterigmatocystin, and prednisolone, and only weakly responsive to DTIC and mitolactol. However, M109 was found to be sensitive to several "P388-negative" compounds (such as 6-thioguanine, 6-mercaptopurine, bleomycin, and pyran) and thus, with cognizance of its limitations, M109 might be considered for screening such agents.

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Long-Term Chemotherapy-Induced Remission in a Probable Case of Hairy Cell Leukemia[1,2]

Brief Reports and Preliminary Communications

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Hairy cell leukemia was first described in 1958 and is still frequently misdiagnosed as acute leukemia, chronic lymphocytic leukemia, or lymphoma (1,2). Splenectomy, the treatment of choice (3), usually leads to amelioration of pancytopenia but improvement only persists in about one third of all patients (4). Once bone marrow failure ensues, treatment options are limited. The chemotherapy for hairy cell leukemia has recently been reviewed (5), and only a handful of favorable responses have been described following treatment with chemotherapy or androgen therapy.

We recently reviewed the bone marrow aspirations of our 16 patients with acute lymphocytic leukemia who had been in complete remission for ≥ 5 years. The bone marrow morphology of one of these patients was highly suggestive of hairy cell leukemia. His case history is presented below.

CASE REPORT

Patient 1

In July 1970, patient 1, a 42-year-old rancher, presented with persistent fever, malaise, arthralgias, weight loss, granulocytopenia, lymphocytosis, and splenomegaly. A splenectomy and liver biopsy were performed and a diagnosis was made of acute lymphocytic leukemia infiltrating liver, spleen, and lymph nodes. Preoperative blood cell counts showed a hemoglobin level of 13.3 g/dl, a platelet count of 100,000/μl, and a wbc count of 3100/μl, with 37% neutrophils and 63% lymphocytes. The patient was transferred to the M. D. Anderson Hospital and Tumor Institute at which time his hemoglobin level was 11.6 g/dl, his platelet count was 425,000/μl, and his wbc count was 4000/μl, with 59% neutrophils, 39% lymphocytes, 2% esosinophils, and no monocytes. Bone marrow aspiration was performed with difficulty and revealed 66% leukemic cells, 3.6% granulocyte precursors, 5.4% neutrophils, 1.0% eosinophils, 10.6% lymphocytes, 0.7% plasma cells, 13% normoblasts, decreased megakaryocytes, and a cellularity of 90%. "Frayed cytoplasm" was noted in some leukemic cells.

Four courses of chemotherapy using the COAP regimen (cyclophosphamide and cytarabine [100–200 mg/m²/day for 5 days each], prednisone [200 mg/day for 5 days], and vincristine [2 mg on Day 1 only]) were required to achieve a complete remission. A sterile laminar air flow room ("protected environment") and prophylactic systemic antibiotics were used. The patient had one episode of pneumonia and one episode of fever of unknown etiology requiring intensive antibiotic therapy.

Five consolidation and maintenance courses of chemotherapy were then given with doses of cyclophosphamide and cytarabine varying from 100 to 50 mg/m²/day. Four of the five courses were accompanied by infections requiring systemic antibiotics. The patient then received two courses of 6-mercaptopurine and methotrexate, two additional courses of cyclophosphamide and cytarabine, and three courses of daunorubicin followed by 12 additional courses of COAP, without significant complications. Immunotherapy with BCG was begun in December 1972 and was continued until October 1975. At last followup in November 1978, the patient remained in complete remission.

Original bone marrow aspiration slides from 1970, as well as histopathology of spleen, liver biopsy, and lymph nodes, were reviewed in January 1979 by hematopathologists who felt that the most likely diagnosis was actually hairy cell leukemia. No tissue was available for tartrate-resistant acid phosphatase staining or for electron microscopy.

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DISCUSSION

Hairy cell leukemia is frequently a difficult diagnostic problem (6). In the case presented here, examination of tissue and bone marrow aspiration slides by skilled hematopathologists strongly suggested the diagnosis of hairy cell leukemia; however, because of the age of the specimens and the lack of unfixed and unstained materials, it was impossible to perform tartrate-resistant acid phosphatase staining (7) or electron microscopy (8) to confirm the diagnosis. Since the patient remains in complete remission, no fresh malignant cells are available for further testing; therefore, an unequivocal diagnosis of hairy cell leukemia cannot be made. Nevertheless, the clinical presentation was quite compatible with this diagnosis, with pancytopenia (including severe monocytopenia) and splenomegaly. Moreover, his disease was not characteristic of acute lymphoblastic leukemia since very few adult patients with that malignancy remain in long-term complete remission (9), whereas even a partial remission may be reasonably durable in hairy cell leukemia (5,10). Thus, in view of the pathologic findings and the clinical presentation, we feel that the patient actually did have hairy cell leukemia.

A single complete remission in hairy cell leukemia has previously been reported with the combination of cyclophosphamide and cytarabine (11). This remission persisted for at least 2 years despite the fact that the patient received only one course of treatment. Our case can be interpreted as *probable* supportive evidence of the efficacy of this drug combination in hairy cell leukemia. These data are important since treatment options are limited in hairy cell leukemia once bone marrow failure occurs. We also have two additional patients with a probable diagnosis of hairy cell leukemia who have been in complete remission for 10 and 8 years, respectively, after treatment with a cyclophosphamide and cytarabine combination in the first patient and a combination of daunorubicin, cytarabine, vincristine, and prednisone induction followed by cyclophosphamide and cytarabine maintenance therapy in the second patient. Both patients required multiple courses of chemotherapy and intensive supportive care to achieve remission. They had initially been diagnosed as having acute lymphoblastic leukemia, but in retrospect had clinical presentations, courses, and bone marrow aspirates strongly suggestive of hairy cell leukemia. One patient also had a pretreatment bone marrow biopsy, and on review it appears to be characteristic of hairy cell leukemia. One year after attaining bone marrow remission, this patient underwent a splenectomy for hypersplenism. The spleen showed only granulocytic metaplasia at that time, although a splenic hilar lymph node appears to have had residual hairy cell leukemia. Unfortunately, since pretreatment splenic pathology cannot be examined in either of these last two patients, a diagnosis of hairy cell leukemia would be even more tenuous than in our case presented above.

Our patient's splenectomy may have contributed to his favorable clinical course, although splenectomy will usually just ameliorate pancytopenia without directly affecting the amount of disease in the bone marrow. Splenectomy remains the initial treatment of choice for hairy cell leukemia (3), but only one of three patients will gain sustained benefit from this procedure (4), and most will eventually require alternative therapy since bone marrow failure occurs secondary to infiltration by leukemic cells.

The 6-mercaptopurine, methotrexate, daunorubicin, and BCG given during maintenance therapy may also have contributed to the long duration of our patient's remission. The chemotherapy of hairy cell leukemia has recently been reviewed (5). Generally, only transient hematologic improvement has been noted with alkylating agents, corticosteroids, vinca alkaloids, and androgens. Nevertheless, remissions or substantial benefit have been noted with anthracyclines alone or in combinations, cytarabine-containing combinations, a combination of cyclophosphamide, vincristine, and prednisone (5), and continuous low-dose chlorambucil (10). Improvement has also been noted following prednisone alone or combined with vincristine or etiocholanolone and with oxymetholone alone (5). Amelioration of the immune defect has been noted following mononuclear cell transfusion (12).

In summary, we have presented the case of a patient who probably had hairy cell leukemia although it is impossible to unequivocally confirm the diagnosis. He achieved long-term complete remission when he was treated with cyclophosphamide, cytarabine, vincristine, and prednisone, a combination which has previously been noted to be of benefit in hairy cell leukemia (11). While we do not recommend chemotherapy as initial treatment in hairy cell leukemia, once it becomes necessary, high doses of drugs such as cyclophosphamide and cytarabine-based combination coupled with intensive supportive care may enable successful induction of long-term remission.

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CAMP (Cyclophosphamide, Doxorubicin, Methotrexate, and Procarbazine) for Epidermoid and Large Cell Anaplastic Carcinoma of the Lung[1]

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Bronchogenic carcinoma is the leading cause of cancer death in the US, accounting for approximately 93,000 deaths/year (1). It ranks first as a cause of cancer-related deaths in men and ranks second in women (2). More than 50% of patients with bronchogenic carcinoma present with metastatic disease (3) and are therefore inoperable and potential candidates for chemotherapy. Whereas response rates and survival for patients with small cell cancer of the lung have shown substantial improvements with aggressive chemotherapeutic regimens (4), the non-small cell bronchogenic carcinoma (NSBC) remains relatively resistant to both chemotherapy and radiation therapy. Recently, Bitran et al (5) reported a 50% response rate in NSBC using a combination of cyclophosphamide, doxorubicin, methotrexate, and procarbazine (CAMP), which decreased to 33% with further followup (6). We report here our experience with the CAMP regimen.

PATIENTS AND METHODS

From November 1976 to September 1979, 35 consecutive patients with a histologic diagnosis of NSBC were treated with CAMP. Patients who had had previous chemotherapy were excluded, and patients with adenocarcinoma were included in a previous study employing 5-FU, doxorubicin, and mitomycin C (FAM) (7). The clinical characteristics of the study population are presented in table 1. Twenty patients had epidermoid carcinoma, and 15 had large cell anaplastic carcinoma. Twenty were male and 15 were female. Twenty-five patients received radiation therapy: nine for cerebral metastases, six for symptomatic bone disease, and ten for intrathoracic complications and palliation of pain. Twelve patients had disease localized to the thorax, and 23 had extensive disease. Performance status (PS) was assessed by the Eastern Cooperative Oncology Group (ECOG) scale (PS 0, asymptomatic; PS 1, ambulatory with symptoms; PS 2, out of bed $\geq$ 50% of the day; PS 3, out of bed $<$ 50% of the day; PS 4, fully bedridden). Three patients had a PS of 0, 11 had a PS of 1, 15 had a PS of 2, and six had a PS of 3.

Patients were treated with CAMP in the usual doses described by Bitran et al (6): cyclophosphamide (Cytoxan), 300 mg/m^2 iv on Days 1 and 8; doxorubicin, 20 mg/m^2 iv on Days 1 and 8; methotrexate, 15 mg/m^2 on Days 1 and 8; and procarbazine, 100 mg/m^2 orally on Days 1–10. In patients initially treated with radiation therapy, chemotherapy was begun approximately 2 weeks after the completion of irradiation. Full doses were administered for a wbc count $>$ 3500/mm^3 and a platelet count $>$ 100,000/mm^3. For a wbc count between 2500 and 3500/mm^3 and/or a platelet count between 75,000 and 100,000/mm^3, the dose was reduced by 50%.

At least one full cycle of CAMP was required for patients to be evaluable. Response criteria were defined as follows: *complete response*—the disappearance of all measurable disease; *partial response*—a $>$ 50% reduction in measurable lesions (product of the greatest diameter and its perpendicular) without disease progression in other sites for at least 2 months; *stable disease*—a $<$ 50% reduction or no disease progression for at least 2 months; and *no response*—progressive disease or the appearance of new lesions. Disease in irradiated ports was not considered measurable. Response by bone scan criteria was noted by a decrease in the number of lesions; a decrease in intensity was judged as stable disease. A response in the liver was defined as a $>$ 30% reduction of total span as determined by physical examination, and by the disappearance of lesions on scan. Diminution of focal scan abnormalities was considered as stable disease.

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TABLE 1.—Clinical characteristics of CAMP-treated patients

Patient No.	Age (yrs)	Sex	Pathology*	Extent of disease†	Extrapulmonary sites of disease	Site of radiotherapy	Response‡	Response duration (mos)	Survival (mos)	ECOG PS
					Patients with measurable disease					
1	54	M	EPI	E	Brain	Brain	PR	4	6	0
2	67	M	EPI	E	Retroperitoneum		PR	4	8	1
3	47	M	LG	E	Bone	Bone	PR	6	8+	1
4	42	F	LG	L			CR	20+	24+	0
5	59	M	EPI	E	Bone	Bone	S		14+	1
6	63	M	EPI	L			S		10	1
7	61	M	LG	E	Brain Retroperitoneum	Brain	S		6	3
8	59	M	EPI	L			S		6+	1
9	67	F	LG	E	Brain	Brain	S		6	2
10	59	F	LG	E	Brain Bone	Brain	S		4+	1
11	63	M	LG	E	Bone	Bone	S		11+	1
12	79	M	EPI	E	Brain Bone	Brain Bone	NR		3	3
13	53	M	EPI	E	Bone Retroperitoneum	Bone	NR		3	2
14	49	M	EPI	E	Bone Liver	Bone	NR		4	2
15	50	M	EPI	E	Bone		NR		7	2
16	57	M	EPI	E	Bone	Bone	NR		2	3
17	64	F	LG	L			NR		4	2
18	59	M	EPI	E	Liver		NR		5	2
19	74	F	EPI	E	Bone		NR		4+	2
20	64	F	LG	L			NR		2	3
21	62	F	LG	E	Brain	Brain	NR		4	3
22	63	F	LG	E	Brain	Brain	NR		4+	1
23	53	F	LG	L			NR		2	2
					Patients without measurable disease					
24	51	M	EPI	E	Brain	Brain			12+	0
25	62	M	EPI	E	Brain	Brain			9	2
26	63	F	LG	E	Bone Liver	Lung			6	2
27	63	M	LG	L		Lung			12+	1
28	65	M	LG	E	Appendix	Lung			4+	1
29	66	M	EPI	L		Mediastinum			3	2
30	68	F	EPI	L		Mediastinum			2	2
31	49	M	EPI	L		Lung			2	1
32	55	F	EPI	E		Lung			2	2
33	49	F	EPI	E	Pericardium	Pericardium			4+	2
34	60	F	EPI	L		Lung			2	3
35	53	F	LG	L					2	2

*EPI = epidermoid carcinoma, LG = large cell carcinoma.
†E = extensive, L = limited.
‡PR = partial response, CR = complete response, S = stable disease, NR = no response.

Survival was calculated from the initiation of chemotherapy, and the cumulative survival rate was determined by the life-table method of Cutler and Ederer (8). Life-table curves were compared by means of the chi-square test for statistical analysis (9).

RESULTS

Of 35 patients in the study, 23 had measurable disease. Twenty patients had measurable pulmonary lesions, and three had measurable bone metastases. The remaining patients were not evaluable because radiation therapy was administered to the indicator lesions. Four tumor responses were noted, giving a response rate of 17% (four of 23 patients). Two patients with epidermoid carcinoma responded, one with a partial decrease in pulmonary lesions and the other with a partial decrease in a retroperitoneal tumor. The duration of response was 4 months for each. Two patients with large cell anaplastic carcinoma had pulmonary responses. One patient had a partial response of 6 months and the other had a complete response of > 20 months.

Seven of 23 patients were classified as having stable disease (30%). Survival is shown in figure 1. The median survivals were 8 months for responders, approximately 4 months for nonresponders, and approximately 10 months for patients with stable disease. The entire group of patients had a median survival time of approximately 6 months. When responding patients and those with stable disease were grouped together and compared to nonresponders, the difference in survival was highly significant ($P < 0.01$). Those patients achieving a partial response or disease stabilization had a median PS of 0–1 (range, 0–2), whereas nonresponding patients had a median PS of 2–3 (range, 1–3). These differences were also significant ($P < 0.01$).

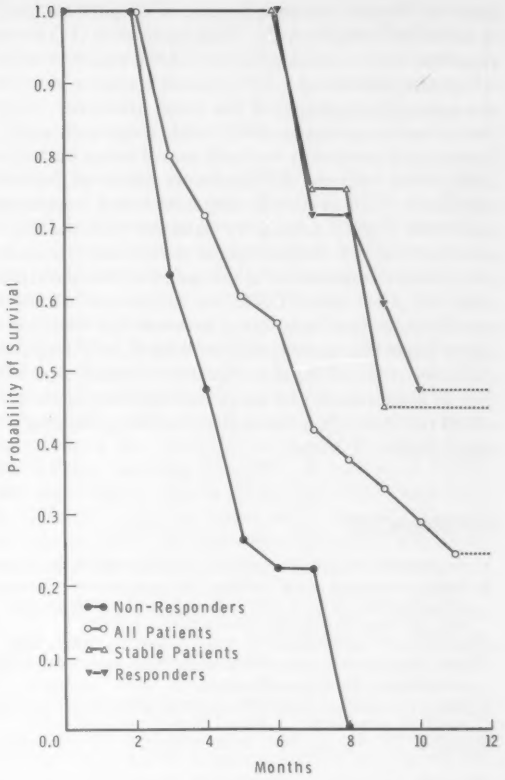

FIGURE 1.—CAMP in epidermoid and large cell bronchogenic carcinoma.

DISCUSSION

The initial enthusiasm for combination chemotherapy in the treatment of NSBC has diminished since early reports of high response rates have not been confirmed with continued followup. For example, the updated response rate of the BACON regimen (bleomycin, doxorubicin, CCNU, vincristine, and mechlorethamine) in epidermoid carcinoma was 22% (10) in contrast to the initial response rate of 45% reported in an earlier trial (11). Similarly, the combination of methotrexate, doxorubicin, cyclophosphamide, and CCNU had been reported as producing a 36% response rate among 22 patients with epidermoid lung cancer (12); the ECOG study of this combination, however, reported only four responses among 27 patients (15%) who were reasonably well-matched to those in the earlier trial in terms of PS and extent of disease (13).

Our experience with the CAMP program in epidermoid and large cell bronchogenic carcinoma is consistent with these findings; we were unable to confirm the encouraging reports by Bitran et al (6), although we adhered completely to their dose recommendations as well as to their criteria of response. Our patients appear to be comparably matched in prognostic factors with those of Bitran et al; the mean age was 63 years (range, 35–78) in Bitran's series and 59 years (range, 42–74) in our population. Twenty-one of Bitran's 61 patients had a good PS (ie, a Karnofsky PS of 70) compared to 14 of our 35 patients with an ECOG PS of 0–1. Nonetheless, only two of our 12 patients with epidermoid carcinoma and two of 11 with large cell anaplastic carcinoma of the lung responded, in con-

trast to Bitran's success in nine of 33 patients and in one of 12, respectively. Vogelzang et al (14) have reported their results with the CAMP regimen with which they obtained a 31% overall response rate in non-oat cell carcinoma of the lung. However, only two of seven patients (28%) with large cell undifferentiated carcinoma and only one of seven patients (14%) with epidermoid carcinoma achieved partial responses. Lad et al (15) obtained a 28% response rate with CAMP among 51 patients with measurable non-oat cell bronchogenic carcinoma. In their preliminary analysis of a prospective comparative trial of CAMP and CCNU, no difference in treatment results has been found between the two therapies. Based on our experience with CAMP and the above-mentioned studies, we recommend that effort in epidermoid and large cell histologies be devoted to identifying the activity of drugs in single-agent phase II trials.

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Hepatic Toxicity of DTIC[1]

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DTIC is in widespread use for the treatment of metastatic malignant melanoma. The most common toxic effects of DTIC are gastrointestinal, with nausea and vomiting being frequent manifestations, and hematologic, with mild leukopenia and thrombocytopenia seen in the doses commonly used. This case report describes a patient who developed DTIC-induced hepatocellular dysfunction with severe hematologic toxicity.

CASE REPORT

Patient 1

Patient 1 is a 43-year-old white female who developed a primary malignant melanoma of the right fourth toe in October 1975. She was treated by amputation of the digit, common femoral perfusion with melphalan, and inguinofemoral lymph node dissection that yielded one positive node. She developed a subcutaneous nodule of melanoma in the upper thigh in March 1978; resection of the nodule was followed by an iliac perfusion with 26 mg of mechlorethamine. The patient developed another subcutaneous nodule in her right leg in December 1978 which was locally excised. All studies at that time, including liver enzymes and a liver-spleen scan, were within normal limits. From January 8 to 12, 1979, the patient was given a course of systemic chemotherapy composed of 270 mg of DTIC (2.7 mg/kg) iv on Days 1–5, 2 mg of vincristine iv on Days 1 and 5, and 150 mg of CCNU (1.5 mg/kg) orally on Day 2. Trifluprom azine was used for nausea in this and in subsequent courses. No toxic effects occurred. Liver function studies on March 5, 1979, were within normal limits. A repeat course was given between March 4 and 8, 1979. Again, no unusual toxic effects were noted. A third course of systemic chemotherapy composed of 350 mg of DTIC iv on Days 1–5, 1 mg of vincristine on Days 1 and 5, and 120 mg of CCNU on Day 2 was administered between April 16 and 20, 1979. On May 3, 1979, bilirubin was elevated at 1.2 mg/dl (normal, 0.2–1.2) and SGOT was elevated at 58 IU/liter (normal, 9–41), while LDH and alkaline phosphatase were within normal limits. Hematologic toxicity was noted in this course, with a wbc count nadir of 2400/mm^3 on May 18, 1979, and a platelet count nadir of 48,000/mm^3 on May 14, 1979. On June 27, 1979, two new nodules of melanoma in the right leg were locally excised. All laboratory studies were normal with the exception of the LDH, which was 230 IU/liter (normal, 105–225). A course of DTIC (400 mg iv/day × 5) was given June 24 through July 3, 1979. The platelet count nadir was 17,000/mm^3 on July 26, 1979, and the wbc count nadir was 2500/mm^3 on August 3, 1979. Hematologic recovery occurred by August 27, 1979, and another cycle of DTIC (400 mg iv daily × 5) was given. The platelet count nadir occurred on September 18, 1979, at 9000/mm^3, with gross bleeding requiring eight units of platelets. Wbc count nadir occurred on September 26, 1979, at 1500/mm^3. The patient noted itching and jaundice and was admitted to the hospital on November 5, 1979. Admission laboratory work revealed a wbc count of 6300/mm^3, adequate platelet count, and a total bilirubin of 6.2 mg/dl with a direct fraction of 4.3 mg/dl. Alkaline phosphatase was 260 IU/liter, LDH was > 600 IU/liter, SGOT was 576 IU/liter, and SGPT was 402 IU/liter. A liver-spleen scan was interpreted as normal. A computerized axial tomographic scan and an ultrasonogram of the abdomen were both within normal limits. Hepatitis A antibody and hepatitis B surface antigen determinations were both negative. A liver biopsy was performed on November 13, 1979. The histologic sections show swollen and pleomorphic parenchymal cells. These changes are more prominent in the centrilobular region. Intracellular cholestasis and lipofuscin pigment are observed. The Kupffer's cells are hyperplastic and contain PAS-positive material. Few lymphocytes are observed among the hepatic cells. The portal spaces show some lymphocytes, polymorphonuclear leukocytes, and eosinophils. There are also parenchymatous cells showing fatty metamorphosis and vacuolated nuclei.

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All liver function studies returned to normal on December 27, 1979. On January 4, 1980, a single dose of DTIC (200 mg) was given alone with no medication. On January 10, 1980, the total bilirubin was 1.5 mg/dl, the alkaline phosphatase was 90 IU/liter, the LDH was elevated at 303 IU/liter, and the SGOT was elevated at 195 IU/liter. The bilirubin returned to normal by January 18, 1980, but enzymes remained elevated 4 weeks after DTIC administration.

DISCUSSION

The common toxic effects of DTIC, composed of nausea and vomiting, leukopenia, and thrombocytopenia, are well-known. It was reported in some phase I and II studies of DTIC that some patients developed transient elevations in SGOT with DTIC administration. No serious hepatic toxicity was noted (1–3). Recently, several severe episodes of hepatic toxicity have occurred in patients receiving therapy that included DTIC (4) (Dome Division, Miles Laboratories; unpublished data). These cases have been difficult to evaluate since multiple drugs were used, some or all of which are known to produce hepatic toxicity. This case clearly demonstrates that not only can elevations of enzymes occur, but serious hepatic dysfunction with jaundice and histologic change can occur as a result of DTIC administration.

Triflupromazine is hepatotoxic in man and CCNU is hepatotoxic in animals. Elevations of liver function values occasionally occur while patients are receiving CCNU (5). However, CCNU is almost always administered with other drugs, making it difficult to determine its hepatotoxicity in man. The role of these drugs in sensitizing the patient to DTIC is unknown.

Another of the less common but equally serious complications is the occasional appearance of severe, unexplained hematologic toxic effects while patients are being treated with standard doses of DTIC, which some authors have labeled an "idiosyncratic" type reaction (3). In this case, leukopenia and severe thombocytopenia occurred at a time when minor elevations in hepatic enzymes were seen. It may well be that the idiosyncratic reaction seen with DTIC occurs in those patients in whom DTIC is causing hepatocellular dysfunction. The liver seems to be the site of DTIC metabolism (6). An alteration in liver function may well increase the half-life of the active components of this drug, causing seriously increased toxicity.

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Physiologic Response and Toxicity in Patients Undergoing Whole-Body Hyperthermia for the Treatment of Cancer[1]

Brief Reports and Preliminary Communications

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SUMMARY

Seven patients with advanced cancer underwent whole-body hyperthermia using a nylon and vinyl mesh, water-perfused suit. Treatments were given at 41.8°C for 4 hours. Five patients received concomitant cyclophosphamide with hyperthermia. Compared to baseline (37°C) conditions, there was a significant rise in pulse rate ($P < 0.001$), a fall in diastolic pressure ($P < 0.02$), and an increase in respiratory rate ($P < 0.001$). Toxic effects included fatigue, extremity edema, diarrhea, nausea and vomiting, and respiratory depression in a patient with cerebral metastases. Compared to baseline values, there was a significant increase in serum glucose ($P < 0.02$) and decreases in serum calcium ($P < 0.01$) and phosphorus ($P < 0.01$). Significant elevations in serum LDH and SGOT values occurred 24 hours following hyperthermia, suggesting hepatic sensitivity to heat. The methods used to induce whole-body hyperthermia, as described in this paper, are feasible, permit relatively easy access to the patient, and are potentially applicable in diverse hospital settings such as intensive care units, radiation therapy areas, and conventional rooms. The physiologic alterations that were observed and the toxic effects that were documented indicate that careful monitoring of patients is necessary.

[Cancer Treat Rep 65:323–325, 1981]

Whole-body hyperthermia (WBH) using various methods including diathermy, melted paraffin wax with heated anesthetic gases, and modification of twin hypo-hyperthermia hospital blankets has been employed in a variety of tumors (1–5). Disadvantages of these methods have included the necessity of general anesthesia, limited access to the patient undergoing WBH, and the lack of mobility of the equipment used. The purposes of this paper are to describe a method of achieving WBH which provides a relatively easy means of studying the physiologic responses to it and to report the results from our first seven patients with advanced cancer who have undergone this experimental treatment.

METHODS

Any patient with a histologically documented diagnosis of cancer refractory to conventional therapy was considered for WBH. Informed consent was obtained prior to therapy.

The patient is dressed in a full-body plastic suit (Dynamics Classics, Ltd, Fairfield, NJ). Rectal and esophageal probes are attached to an electronic thermometer (Model BAT-8, Bailey Instruments, Saddlebrook, NJ) to give a continuous readout of core temperature.

Core body temperature is then slowly elevated by the use of a Flexitherm nylon and vinyl mesh, water-perfused suit (Acurex Corp, Mountain View, CA), which is connected by hose to a thermal conditioning unit (Acurex Corp) supplied by the National Aeronautics and Space Administration. Five percent dextrose in 0.45 N saline with 10 mEq of potassium chloride is given by iv infusion at a rate of 500–1000 ml/hour. At the termination of treatment, the patient is cooled by opening the insulating suit and lowering the water flow temperature to 37°C.

Patients are sedated during WBH with sodium thiopental at doses of 50–150 mg given by iv bolus. Once the desired temperature is achieved, patients frequently do not require any more sedation even

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TABLE 1.—Vital signs measured before, during, and 1 hr after WBH*

	Pretherapy	41.8°C minus pretherapy values	1 hr after WBH minus pretherapy values
Pulse (min $^{-1}$)	105 (80–140)	60 (−20–80)	12 (−20–68)
Respirations (min $^{-1}$)	24 (20–36)	30 (16–40)	8 (−12–20)
Systolic pressure (mm Hg)	110 (84–144)	20 (−10–40)	−16 (−45–22)
Diastolic pressure (mm Hg)	70 (42–88)	−10 (−26–0)	−18 (−30–20)

*Median differences (range) between values at 41.8°C or 1 hr after end of therapy and pretherapy values measured in each patient.

TABLE 2.—Serum chemistries*

	Pretherapy	41.8°C minus pretherapy values	24 hrs after WBH minus pretherapy values
Glucose (mg/dl)	95 (80–129)	79.5 (33–242)	−14 (−48–8)
Potassium (mg/liter)	4.2 (3.7–4.6)	−0.1 (−0.6–0.8)	−0.4 (−0.8–−0.1)
Calcium (mg/dl)	9.2 (8.7–9.9)	−1.1 (−1.6–0)	−0.9 (−2.4–−0.4)
Phosphorus (mg/dl)	3.3 (2.1–3.9)	−2.0 (−3–0.4)	−0.2 (−1.4–0.6)
CO_2 (mEq/liter)	25 (21–37)	−3 (−13–0)	−6 (−13–−2)
Alkaline phosphatase (mu/ml)	77 (43–107)	3 (−7–75)	−7 (−14–4)
SGOT (milliunits/ml)	16.5 (11–36)	6 (0–33)	18.5 (3–53)
LDH (milliunits/ml)	189 (91–370)	20 (−14–83)	100 (−18–955)

*Median differences (range) between values at 41.8°C or 24 hrs following WBH and pretherapy values measured in each patient.

though the procedure may last an additional 4–6 hours. The median dose given per course was 2065 mg (range, 800–3600).

Statistical analysis was performed using a multiple-comparison procedure associated with Friedman's nonparametric two-way analysis of variance (6). For each parameter, the readings for a patient before, during, and after WBH are considered observations from the same distribution (with the same median). The significance levels are of the two-sided type.

RESULTS

Four patients had carcinoma of the lung, two had pleural mesothelioma, and one had an abdominal fibrosarcoma. The median age was 58 years (range, 47–62). Five patients had a performance status of 1 (ambulatory with some symptoms), and two had a performance status of 3 or 4 (in bed > 50% of the time).

Five of the seven patients received cyclophosphamide concomitant with WBH for the purpose of evaluating the effect of hyperthermia on cyclophosphamide pharmacokinetics.

The physiologic and laboratory measurements for treatments given at 41.8°C for 4 hours (total, 13 courses) are summarized in tables 1 and 2 which show medians and ranges of the pre-WBH values and of the subsequent changes relative to the baseline.

During WBH, the pulse rate increased substantially over pretreatment values ($P < 0.001$) and remained elevated at 1 hour after therapy; it returned to baseline values by 24 hours following therapy. The diastolic pressure during WBH at 41.8°C and at 1 hour following treatment showed significant changes compared to pretreatment values ($P < 0.02$ and $P < 0.005$), respectively). The respiratory rate markedly increased during treatment in all patients ($P < 0.001$ at 41.8°C).

The serum glucose was significantly elevated (table 2) during WBH in all patients ($P < 0.02$) but returned toward baseline values 24 hours after therapy. Calcium ($P < 0.01$) and phosphorus ($P < 0.01$) were significantly decreased during WBH, but increased urinary losses were not documented. Potassium ($P < 0.02$) and serum bicarbonate ($P < 0.001$) were significantly depressed 24 hours after treatment. There was some elevation in liver function

tests (table 2) during WBH but the only significant elevations were the LDH and SGOT values 24 hours following treatment ($P < 0.04$ and $P < 0.001$, respectively).

Toxic Effects

Fatigue, arm and leg edema, and diarrhea were observed in all patients and usually resolved within 48 hours following therapy. One patient developed apnea during the third hyperthermia treatment and died 24 hours after termination of the procedure. A necropsy revealed extensive pulmonary and brain metastases including the brainstem, but there was no evidence of massive tumor necrosis. A computerized tomographic scan of this patient's brain was normal before the WBH treatments began. Two other patients had documented brain metastases and tolerated WBH without major complications. Although it is unclear if there is a relationship between brain metastasis and increased toxicity during WBH, we no longer use WBH to treat patients with brain metastasis.

DISCUSSION

The purpose of this study was to determine the feasibility of WBH and the physiologic and toxic responses to it, using a new method in patients with advanced cancer. In general, the treatments were reasonably well-tolerated by the patients.

An elevation in serum glucose was seen in our patients during hyperthermia and may reflect the infusion of 5% dextrose in water and the stress of WBH with release of glycolytic enzymes and endogenous steroids. Hyperglycemia has been reported by others (1). The decrease in phosphorus observed in our patients has also been seen by other investigators and may be secondary in part to respiratory alkalosis and hyperglycemia, both occurring with hyperthermia (1,2,6). Increased urinary phosphorus loss has not been documented, and intracellular shifts have been postulated to account for the low values observed (7,8). Hyperthermia has been reported to cause elevated liver enzymes and the liver is known to be sensitive to heat (5,7). In one report (3), two patients with massive tumor involvement of the liver died following hyperthermia therapy; extensive tumor necrosis was documented at postmortem examinations.

The methods described in this paper to induce WBH in patients with cancer are feasible. These methods allow relatively easy access to the patient and are potentially applicable in diverse hospital settings such as radiation therapy areas, intensive care units when special measurements are needed, and in a conventional hospital room. However, the physiologic alterations observed, as well as changes in laboratory parameters and the toxic effects that were documented, suggest that this treatment requires careful monitoring of patients.

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Unanticipated Side Effects From Treatment With High-Dose Mechlorethamine in Patients With Malignant Melanoma[1]

Brief Reports and Preliminary Communications

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Standard doses of alkylating agents are not very active in patients with advanced malignant melanoma (1). High doses of alkylating agents have, however, produced tumor responses in patients with nonresponsive and/or disseminated disease (2,3). Based on this finding, we began (in December 1979) a treatment study using high-dose mechlorethamine preceded by bone marrow procurement and followed by bone marrow autograft. Three patients with advanced malignant melanoma were treated between December 1979 and February 1980. In addition to the well-known side effects of mechlorethamine, ie, nausea and anorexia, bone marrow suppression, thrombophlebitis at the site of the infusion, alopecia, hepatotoxicity, and immunosuppression, we observed two previously undescribed side effects, ie, cardiotoxicity and hypocalcemia.

The outline of the protocol was as follows. After determination that the bone marrow was disease-free, patients were taken to the operating room where 20–25 ml/kg of bone marrow was removed by multiple aspirations from the posterior iliac crest and sternum. As soon as the patient was awake following surgery, 33 mg/m^2 of mechlorethamine (between 0.7 and 0.9 mg/kg) was given as a slow iv push over 45–60 minutes. The bone marrow was stored at 4°C and was reinfused iv 48 hours after treatment with mechlorethamine. Details of this protocol will be reported elsewhere.

Three patients were studied after written informed consent was obtained as approved by the Human Subjects Committee, University of Colorado Health Sciences Center. There were one female and two male patients, 17, 46, and 52 years of age, respectively. All three patients had stage III disease on entry in the protocol. The primary lesions were excised 3, 19, and 36 months prior to this treatment. Measurable disease was present in two patients. One patient had received previous treatment with two courses of DTIC (400 mg × 5 days) and *Corynebacterium parvum* without response and another patient had received local radiation therapy. Neither patient had a history of cardiac disease or hypocalcemia.

RESULTS

Severe nausea and vomiting was noted in all three patients. Two of three patients had mild anorexia, and one had severe anorexia lasting for 7 days and had to receive total parenteral nutrition. All patients had thrombosis and thrombophlebitis at the site of the mechlorethamine injection; this was mild in one patient and severe in the other two, both of whom still have evidence of a clotted venous system from the site of the injection to the shoulder. Diarrhea was mild and was found in only one patient. Hepatotoxicity, manifested by elevated SGOT, SGPT, alkaline phosphatase, and bilirubin levels, was mild and occurred in all three patients. All patients developed mild alopecia and one had mild esophagitis. All patients complained of headache, which was severe in two with signs of meningeal irritation and mild in one. All patients developed severe granulocytopenia (wbc count < 1000/mm^3) and thrombocytopenia (platelet count < 50,000/mm^3). The duration of granulocytopenia lasted for 5 days and the duration of thrombocytopenia lasted for 8 days. Recovery from severe granulocytopenia and thrombocytopenia produced by a standard dose of 0.2 mg/kg of mechlorethamine given for 2 days was > 2 weeks (4). The above-mentioned side effects were anticipated and treated by prophylactic and therapeutic antibiotics, antiemetics, autologous bone marrow transplantation, and, if necessary, blood and platelet transfusions.

Unexpected side effects were tachycardia, clinical and ECG evidence of cardiotoxicity, and hypocalcemia. All three patients had significant tachycardia during the first 4 days, which was not

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associated with elevated temperature. In the three patients, pulse rates peaked at 120, 128, and 132 beats/minute, respectively. The two male patients had pulse irregularities and ECG showed junctional or atrial ectopic beats. In one patient ECG readings just prior to entry in the protocol showed possible evidence of mild right ventricular enlargement but no alteration in rhythm and conduction. Cardiotoxicity was not manifested by decreased voltage, pericardial effusions, elevations in CPK, or congestive heart failure in any of the three patients.

Mild hypocalcemia occurred in all three patients with the lowest levels being 7.5, 8.3, and 8.4 mg/dl, respectively, and was not associated with blood transfusions, dilution of the serum by crystalloid infusions, hyperphosphatemia, or alkalosis. Pretreatment serum calcium levels were normal in all three patients (9.0–10.6 mg/dl). The hypocalcemia was first noted on Day 5 in one patient and on Day 13 in the other two. There was no temporal relationship between the hypocalcemia and the occurrence of premature beats. Serum phosphorus levels remained normal in all three patients despite the hypocalcemia.

The two male patients in this study who had measurable disease did not respond with reduction in tumor size. In the third patient, the objective parameters were not adequate to define whether or not response to this therapy occurred.

DISCUSSION

Alkylating agents, particularly cyclophosphamide given in high doses, are known to be cardiotoxic (5). In one study, acute lethal carditis was described in four of 15 patients given cyclophosphamide at a dose of 45 mg/kg for 4 consecutive days when combined with other cytotoxic agents. ECG showed decreased voltage in all four patients (6).

In a second study, five of 30 patients receiving 120 mg/kg of cyclophosphamide showed significant ECG changes, including one patient with anterior-wall myocardial ischemia and four with unspecific T-wave changes. One patient in this study died from diffuse hemorrhagic myocardial necrosis after receiving a single dose of cyclophosphamide of 240 mg/kg. With mechlorethamine, different cardiotoxicity was seen, manifested by tachycardia and premature beats within 4 days after the administration of mechlorethamine. The nature of the effect of mechlorethamine on myocardial tissue and the conducting system is unclear.

Hypocalcemia has not yet been reported as a side effect of standard-dose mechlorethamine therapy and seems to be a feature of high-dose mechlorethamine treatment. The mechanism by which mechlorethamine suppresses the serum calcium level has not yet been determined. Possible mechanisms could be direct toxic action on osteoclasts or partial inhibition of parathyroid hormone.

There are a number of studies currently going on throughout the world using intensive doses of chemotherapy combined with autologous bone marrow rescue in an attempt to achieve greater and longer remissions in a large number of neoplastic diseases. In many of these studies, multiple-drug therapy has been employed, and it is difficult to tell how many of the side effects are due to each of the single agents. The present study used only a single agent, and the effects and side effects noted here can be attributed to this drug exclusively. It is important to note these effects for future studies in which mechlorethamine may be considered as an agent, especially in combination regimens with drugs known to be cardiotoxic or which produce hypocalcemia.

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Phase II Clinical Trial of VP-16-213 in Ovarian Cancer[1,2]

Brief Reports and Preliminary Communications

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During recent years, the advantage of combination chemotherapy over single-agent chemotherapy has been recognized in ovarian cancer, confirming the general trend observed in the treatment of most malignancies. Thus, the need for broadening the number of known active agents has been emphasized. The Cooperative Group for the Study of Ovarian Cancer, of the European Organization for Research on Treatment of Cancer, therefore initiated phase II studies for the testing of new or insufficiently studied agents in ovarian cancer. We report here a trial of VP-16-213 in patients resistant to prior conventional treatment and conclude that the patients showed no benefit from this drug in the conditions of the study. VP-16-213, a semisynthetic podophyllotoxin derivative with antitumor activity in experimental systems (1,2) and man, had been selected in light of earlier reports of antitumor activity in small series of ovarian carcinoma patients (3–5).

MATERIALS AND METHODS

Nineteen patients from six institutions were entered in this study. Ages of patients ranged from 35 to 75 years, with 11 patients < 50 years old. All patients had histologically proven ovarian carcinoma, clinical stage III or IV (International Federation of Gynecology and Obstetrics classification), resistant to or recurrent after prior treatment. Prior treatment included surgery in 16 patients ("curative" in seven and "palliative" in nine), radiotherapy in four, and chemotherapy in all. Melphalan, doxorubicin plus vincristine, doxorubicin plus cyclophosphamide, and doxorubicin plus cyclophosphamide plus 5-FU were the most commonly used drugs. There was a minimum delay of 4 weeks between prior treatment and VP-16-213 therapy. All patients had a normal renal function, a Karnofsky index > 40, an expected survival of > 4 weeks, and measurable lesions. VP-16-213 was administered orally (using either capsules [17 patients] or ampules [two]) at a daily dose of 120 mg/m² for 5 consecutive days every 3 weeks. In case of leukopenia (wbc count < 4000/mm³) or thrombocytopenia (platelet count < 100,000/mm³), treatment was postponed until recovery.

RESULTS AND DISCUSSION

The number of 5-day courses given varied from one to 16 per patient (median, three courses for a total dose ranging from 2250 to 3700 mg). Toxic effects included nausea and vomiting (ten patients), partial alopecia (five), and myelosuppression (all; table 1). In one patient, the second course of VP-16-213 had to be administered iv because of bowel obstruction due to progressive disease. This led to toxic death due to severe myelosuppression (platelet count < 20,000/mm³ and wbc count < 1000/mm³).

Of the 18 patients thus evaluable for response, four experienced no change in their disease (of 2, 2.5, 3, and 3 months' duration), while 13 had disease progression (> 25% increase in lesion measurements in 12 and severe degradation of general condition in one). In one patient, a short (< 1 month) partial remission was observed, characterized by > 50% decrease in tumor measurements.

This trial failed to indicate any significant activity of VP-16-213 as a second-line therapy in ovarian carcinoma. Although the tolerance was good in general, the observed hematologic toxic effects indicate that drug doses were in the active range, thus reinforcing our negative conclusions, which agree with the experience of other groups. In a recent phase II study by the Gynecologic Oncology Group (6), only two partial responses were observed in 24 evaluable patients. In a compilation of data prior to

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[2]This study was conducted by the Cooperative Group for the Study of Ovarian Cancer, European Organization for Research on Treatment of Cancer. The following institutions participated in the study: Clinique Saint-Michel, Brussels, Belgium (*A. P. Maskens*); Centre Cl. Regaud, Toulouse, France (*J.-P. Armand*); Hospital General de Asturias, Oviedo, Spain (*A. J. Lacave*); Institut Jules Bordet, Brussels, Belgium (*R. L. De Jager*); Finseninstitutet, Copenhagen, Denmark (*H. H. Hansen*); and Institut Gustave-Roussy, Villejuif, France (*J.-P. M. Wolff*).

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TABLE 1.—Drug-induced myelosuppression

	No. of patients
Wbc count (cells × 10^3/mm^3)	
3.0–3.9	4
2.0–2.9	6
1.0–1.9	3
< 1.0	1
Total	14
Thrombocyte count (cells × 10^3/mm^3)	
75–99	3
50–74	—
25–49	—
< 25	2
Total	5

those mentioned above, Radice et al (7) reported four partial responses among 40 evaluable patients. Thus, of 82 patients analyzed to date, only seven partial responses have been observed.

Considering that all patients had been heavily pretreated, the possibility that some activity could be obtained in untreated patients cannot be ruled out by the present data. Indeed, it has been reported that second-line chemotherapy in ovarian carcinoma usually produces very low response rates, even with primarily active agents such as melphalan (8), 5-FU (8), doxorubicin (8–11), and hexamethylmelamine (8). With the last two drugs, however, additional studies have indicated higher second-line response rates: five of 12 patients treated with doxorubicin (12) and six of 21 treated with hexamethylmelamine(13). Similar observations were reported with cisplatin, with response rates of 26.5% (14) and 31.5% (15).

The present conclusions may not be valid for other epipodophyllotoxins. Indeed, in their review of epipodophyllotoxin activity in solid tumors, Radice et al (7) noted that VM-26 had induced objective responses, including one complete response, in eight of 20 patients with pretreated ovarian carcinoma.

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Phase I Trial of Combination Therapy With PALA and 5-FU[1,2]

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SUMMARY

PALA is an inhibitor of de novo pyrimidine biosynthesis. Studies combining PALA with 5-FU in experimental models have demonstrated synergistic antitumor activity with only additive toxicity. The phase I study of PALA in combination with 5-FU is described. Sixteen patients received a total of 29 courses of PALA given as a 24-hour infusion daily for 5 days and 5-FU given by iv bolus at the end of each 24-hour PALA infusion. Cycles were repeated at 28-day intervals. Mucositis was dose-limiting when 940 mg/m²/day of PALA was given with 345 mg/m²/day of 5-FU. Diarrhea, skin rash, and myelosuppression (in decreasing order of frequency) occurred but were not dose-limiting. Alopecia occurred in all patients. Objective responses were seen in single patients with large cell carcinoma of the lung, fibrous histiocytoma, and adenocarcinoma of the colon refractory to prior 5-FU therapy. These studies support further phase II evaluation of the PALA and 5-FU combination.

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PALA is a transition-state analog inhibitor of aspartate transcarbamylase, the enzyme catalyzing the second step in the de novo pathway of pyrimidine nucleotide biosynthesis (1). Preclinical studies indicated a unique spectrum of activity, with this agent being most effective against slowly growing experimental tumors such as Lewis lung carcinoma, B16 melanoma, and glioma 26 but having little activity against the cytokinetically more active murine leukemias (2). Phase I clinical trials have established the dose-limiting toxic effects of PALA to be diarrhea and mucositis, without evidence of myelosuppression (3,4). Antitumor activity was limited in these phase I studies; however, phase II trials of PALA are currently under way at a number of institutions. This drug's unique mechanism of action and its lack of associated myelosuppression make PALA an attractive agent for combination with other antimetabolites which alter pyrimidine nucleotide biosynthesis.

5-FU is a pyrimidine analog whose cytotoxic effects are produced by two separate biochemical mechanisms of action, both requiring conversion of 5-FU to an active nucleotide form and both modulated by co-administration of PALA. Fluorouridine 5′-triphosphate (FUTP) is incorporated into RNA, thereby altering RNA maturation and function. 5-Fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP) binds irreversibly to thymidylate synthetase and thereby inhibits conversion of dUMP to TMP and, by depletion of TTP, causes a rapid and marked reduction in DNA synthesis (5). The inhibition of de novo pyrimidine biosynthesis induced by PALA depletes endogenous uridine pools (6) and enhances the RNA effect of 5-FU by increasing incorporation of FUTP into RNA.[5] The PALA-induced depletion of pyrimidine pools may also augment the DNA effect of 5-FU by lowering levels of dUMP, thereby decreasing this normal substrate's competition with FdUMP for binding to the active site of thymidylate synthetase.

The PALA and 5-FU combination has been evaluated in four murine solid tumors: colon carcinoma 26, Lewis lung carcinoma, colon carcinoma 38, and M5076 ovarian carcinoma. In each of these systems PALA and 5-FU were therapeutically synergistic, whereas toxicity was slightly less than additive (7). We report here the results of a phase I trial de-

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[5]Martin DS, Stolfi RL, Sawyer RC, et al. An overview of thymidine. Presented at the 1979 Workshop on Large Bowel Cancer, Houston, TX.

signed to elucidate the clinical toxicity and efficacy of combined therapy with PALA and 5-FU.

MATERIALS AND METHODS

All patients entered in this study had histologic evidence of malignancy which was refractory to standard therapy or for which there was no conventional therapy of any benefit. Informed consent was obtained from all patients. With the exception of one patient with advanced pancreatic carcinoma, all patients had received multiple chemotherapeutic agents and over one half had also received radiotherapy (table 1). No patient had received chemotherapy for at least 30 days prior to entry into this study. All patients had an estimated survival > 8 weeks, and a majority had a performance status on the Eastern Cooperative Oncology Group scale, of ≤ 2 (> 50% ambulatory). All patients had normal or stable hepatic and renal functions.

PALA was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. PALA was prepared in 1-g ampules reconstituted in 10 ml of 0.85% normal saline without preservative. The pH of the ampules was 6.5–7.5. Prior to drug administration the daily dose of PALA was mixed in 0.85% normal saline to a total volume of 1000 ml. This volume was given as a 24-hour constant infusion daily for 5 consecutive days. At the conclusion of each 24-hour PALA infusion, 5-FU was given by iv bolus at a site separate from the PALA infusion. Courses of treatment were repeated at 4-week intervals.

The starting doses were 500 mg/m²/day for PALA and 185 mg/m²/day for 5-FU. This was determined by taking one third of the maximum tolerated dose of each drug when given as a single agent for 5 consecutive days. A decreasing rate of dose escalation was used and, in the absence of dose-limiting toxicity, the doses of both agents were increased according to the dose escalation schedule shown in table 2. At least three patients were treated at each dose level prior to dose escalation. If no toxicity was observed at a particular dose level after 4 weeks, the next dose level was started. Dose escalations were carried out in the same patient if clinical status remained stable and no dose-limiting toxicity was observed.

Prior to the beginning of chemotherapy all patients underwent routine complete history and physical examination. Initial laboratory evaluation included cbc with differential and platelet counts, serum electrolytes, glucose, liver function tests, and a baseline 24-hour creatinine clearance. All measurable disease was documented fully, prior to the start of therapy. A cbc with differential and platelet counts was repeated at weekly intervals during therapy. Prior to each course of therapy, liver function tests, BUN, creatinine, electrolytes, and 24-hour creatinine clearance were repeated.

TABLE 1.—Phase I trial with PALA and 5-FU: patient characteristics

Characteristics	No. of patients
Total	16
Age in yrs	
Mean: 47	
Range: 17–60	
Performance status	
> 2 (nonambulatory)	4
≤ 2 (ambulatory)	12
Prior treatment	
Chemotherapy only	6
Chemotherapy + radiation therapy	9
None	1
Fluorinated pyrimidine therapy	4

RESULTS

Sixteen patients received a total of 29 courses of PALA and 5-FU. Mucositis was the dose-limiting toxic effect. Diarrhea, a characteristic skin rash, and myelosuppression (in order of decreasing frequency) were also seen. In the absence of mucositis, no other toxic effects were observed. Toxic effects are shown in table 3.

Mucositis

One episode of mild mucositis occurred at the second dose level. At the third dose level, seven of 13 courses resulted in moderate mucositis, which was considered dose-limiting. At the fourth dose level, four of five courses resulted in severe mucositis necessitating hospitalization for iv hydration in all four patients. Mucositis began on Days 5–8 of treatment and had a duration of 7–14 days. In general, the more severe the mucositis, the earlier its onset and the longer its duration.

Diarrhea

Diarrhea and abdominal cramps occurred only in association with mucositis of moderate or severe grades. At the third dose level there were two episodes of diarrhea of mild severity and two episodes of moderate severity. At the fourth dose level, three of the four patients with severe mucositis also had severe diarrhea. Diarrhea always paralleled mucositis in onset and duration.

TABLE 2.—Phase I trial with PALA and 5-FU: dose escalation

Dose level	Escalation	Dose mg/m²/day	
		PALA*	5-FU†
1	Starting dose	500	185
2	50%	750	275
3	25%	940	345
4	25%	1175	430

*PALA was given by continuous iv infusion daily for 5 days every 28 days.
†5-FU was given by iv bolus through a separate iv site at the termination of each 24-hour PALA infusion.

TABLE 3.—Phase I trial with PALA and 5-FU: toxicity

Dose (mg/m²/day)		No. of patients	No. of courses	No. of patients with—						
				Mucositis				Diarrhea		
PALA	5-FU			None	Mild*	Moderate†	Severe‡	None	Mild	Severe
500	185	4	4	4	0	0	0	4	0	0
750	275	5	7	6	1	0	0	7	0	0
940	345	10	13	2	4	7	0	8	3	2
1175	430	5	5	1	0	0	4	1	1	3

*Symptomatic mouth sores but not limiting to oral intake.
†Limiting to solid food intake but not to intake of liquids.
‡Totally limiting to oral intake.

Other Toxic Effects

Myelosuppression was infrequent and occurred in only five of the 29 courses administered. Leukopenia occurred on about Day 16 and the leukocyte counts ranged from 500 to 2100/mm³. Thrombocytopenia generally occurred prior to leukopenia, with a platelet count nadir on about Day 13. In those courses which resulted in myelosuppression the platelet count nadirs ranged from 20,000 to 60,000/mm³. Both thrombocytopenia and leukopenia were of < 7 days' duration and did not require specific therapy in any of the patients in this study. A characteristic skin rash consisting of an erythematous plaque-like eruption with a predilection for intertriginous and skin fold areas occurred with variable severity in association with approximately 50% of the courses which resulted in mucositis of any degree. The rash was painless and healed without scarring prior to the next scheduled course of therapy. Alopecia, which was reversible when therapy was discontinued, was observed in all patients who received more than one course of therapy. On one occasion there was perivenous extravasation of 5-FU which produced a severe slowly healing ulcer at the extravasation site. This is of particular interest since, in the absence of PALA, 5-FU can be given sc without local tissue damage. There was no evidence of hepatic, renal, or CNS toxicity in any of the patients entered in this protocol. Although this was a toxicologic study and evaluation of therapeutic activity was not our primary purpose, partial remissions were seen in single patients with fibrous histiocytoma, large cell carcinoma of the lung, and colon carcinoma. Durations of response were 2, 3, and 5 months, respectively.

DISCUSSION

PALA, by virtue of its ability to block de novo pyrimidine nucleotide biosynthesis, is an attractive agent for combination with a variety of pyrimidine antimetabolites such as cytarabine, 5-azacytidine, azauridine, and 5-FU. Experimental data currently exist only for the PALA and 5-FU combination. In several murine solid tumor systems these agents have demonstrated therapeutic synergism with only additive toxicity (7). Other regimens designed to increase incorporation of 5-FU into RNA by adding thymidine and/or uridine to the PALA and 5-FU combination have also demonstrated marked enhancement of therapeutic effect in murine systems (*see* footnote 5).

In this trial we chose a 5-day continuous infusion

schedule of PALA administration because in phase I studies of PALA alone, this method of administration was not associated with neurologic symptoms or the lower gastrointestinal bleeding seen with bolus PALA administration (3,4). PALA was always given for 24 hours prior to 5-FU administration to insure that inhibition of de novo pyrimidine biosynthesis would be in effect at the time of first exposure to 5-FU.

The maximum tolerated dose of the two agents in combination was reached at approximately two thirds of the maximum tolerated dose of each drug when given as a single agent. The dose-limiting toxicity was mucositis; diarrhea and myelosuppression occurred less frequently but always in association with dose-limiting mucositis. Alopecia, skin rash, and a skin slough at the site of 5-FU perivenous infiltration were the other observed toxic effects. No hepatic, renal, or neurologic toxic effects occurred. Objective responses were noted in large cell carcinoma of the lung, fibrous histiocytoma, and adenocarcinoma of the colon. We conclude that PALA (750 mg/m²/day as a continuous iv infusion for 5 days) with 5-FU (275 mg/m²/day by iv bolus at the termination of each 24-hour PALA infusion), given at 28-day intervals, is an appropriate regimen for phase II evaluation. Alternative combinations using PALA and other pyrimidine antagonists should be considered for phase I trial.

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Phase II Study of PALA in Advanced Large Bowel Carcinoma[1,2]

Brief Reports and Preliminary Communications

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PALA is an analog transition-state inhibitor of aspartate transcarbamylase, an enzyme involved in de novo biosynthesis of pyrimidine nucleotides (1). PALA has significant activity against B16 melanoma, sc implanted Lewis lung carcinoma, and a number of other solid tumors in rodents (2–4).[4] A recent phase I trial at our institution demonstrated tolerable toxicity at a dose level of 1250 mg/m²/day for 5 consecutive days repeated at 4-week intervals (5). In that study, the dose-limiting toxic effect was diarrhea, which was most severe in two patients with significant ascites. Occasional dermatitis was seen, but no significant myelosuppression was recorded. The objective of this study was to assess the antitumor activity of PALA in patients with advanced colorectal adenocarcinoma.

PATIENTS AND METHODS

All patients had known colorectal adenocarcinoma with histologic proof of residual, recurrent, or metastatic disease. Each patient had at least one measurable site of known malignant disease to serve as an objective indicator of response to therapy. Hepatomegaly was employed as a measurable lesion if the liver had been proven to contain metastasis and if there was a clearly palpable liver edge extending at least 5 cm below the xyphoid process or costal margin during quiet respiration. Radionuclide liver scans were used to assess response to therapy only if there was a clearly defined defect in isotope uptake measuring at least 5 cm in greatest diameter. Patients who had other lesions without perpendicular diameters that were clearly measurable by a ruler (eg, pelvic or extrarectal masses) and patients with only physiologic abnormalities (eg, ascites, jaundice, abnormal liver enzymes, or CEA determinations) were not included. Other contraindications to patient selection included total disability (Eastern Cooperative Oncology Group [ECOG] performance score of 4), severe malnutrition, nausea or vomiting, serious uncontrolled infection, major surgery within 4 weeks or exploration and biopsy within 3 weeks, and chemotherapy within 4 weeks (8 weeks for nitrosoureas). Because the primary route of excretion of PALA in man is renal (5), no patient whose serum creatinine was > 1.5 mg/100 ml was entered in the study.

The following studies were performed prior to each course of therapy: history, physical examination with measurement of indicator lesion(s), hemoglobin, wbc and platelet counts, chemistry profile (including measurements of SGOT, serum alkaline phosphatase, direct and total bilirubin, and serum creatinine), and chest roentgenogram.

PALA was administered iv in 100 ml of 5% dextrose in water over 10 minutes at a dose level of 1250 mg/m²/day for 5 consecutive days, with treatment repeated at 4-week intervals.

Objective tumor response was defined as at least a 50% decrease in the product of the bidimensional measurements of the lesion chosen prior to treatment as the primary indicator lesion, with no progression of other lesions or new sites of malignant disease. If the liver was the primary indicator lesion, at least a 30% decrease was required in the sum of the linear measurements below the costal margins and xyphoid process. Disease progression was defined as a > 25% increase in the product of the perpendicular diameters of any indicator lesion, a 25% increase in the sum of the distances below the costal margins and xyphoid process if malignant hepatomegaly was the indicator lesion, the appearance of new lesions, significant deterioration in performance status (> 1 level on the ECOG scale), or weight loss of ≥ 10% of body weight. Duration of response was calculated from the first day of therapy to the last day on which the patient met the criteria for response. Interval to disease progres-

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[4]Clinical brochure: PALA (NSC-224131). Prepared by the National Cancer Institute, Bethesda, MD, Dec 1977.

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TABLE 1.—Toxic effects in 32 patients

	No. of patients with reaction	
	Mild or moderate	Severe
Diarrhea	12	1
Dermatitis	17	3
Nausea and/or vomiting	8	0
Stomatitis	5	1
Anorexia	7	0

sion was calculated from the first day of treatment until the first documentation of progressive disease.

Thirty-two patients (20 men and 12 women) were entered in the study. The mean age was 61 years (range, 43–78). Fifteen patients had received no prior chemotherapy, and 17 patients had previously received treatment with one or more cytotoxic agents. All patients were ambulatory and received outpatient chemotherapy (21 had an ECOG performance status of 0 or 1, and 11 had a performance status of 2 or 3). Measurable disease sites included the liver (25 patients), lung (11), abdomen (four), and other sites (five).

RESULTS

One previously untreated patient experienced a partial response at 8 weeks, with dramatic decrease in the size of his liver and subsequent normalization of liver function tests. His partial response has continued for 8+ months. Seventeen patients remained objectively stable for periods ranging from 8 to 28 weeks. Immediate progression was seen in six previously untreated and eight previously treated patients.

Toxicity is reviewed in table 1. Generally, this was transient, mild, and limited to gastrointestinal and mucocutaneous reactions. Stomatitis was classified as severe if it prevented any oral intake of food. Diarrhea was considered severe if it was of sufficient degree to cause dehydration. Dermatitis was maculopapular with subsequent peeling.

Two interesting therapeutic results were noted in associated nonmalignant diseases. One patient had complete clearing of his psoriasis and another stated that he had striking symptomatic improvement of his rheumatoid arthritis. Neither patient was receiving topical or systemic treatment for these diseases. Unfortunately, both of these patients had immediate tumor progression so that they were each treated with only one cycle of PALA.

DISCUSSION

Among 32 patients with advanced colorectal carcinoma treated with PALA in a 5-day course, only one patient (3%) experienced an objective partial response. It would not appear that PALA used alone by our method has a significant potential for treatment benefit in large bowel cancer.

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Phase II Trial of Indicine *N*-Oxide (INDI) in Patients With Advanced Colorectal Carcinoma[1,2]

Brief Reports and Preliminary Communications

W. C. Nichols,* C. G. Moertel, J. Rubin, A. J. Schutt, and J. C. Britell[3]

Indicine *N*-oxide (INDI) is a pyrrolizidine alkaloid first isolated from *Heliotropium indicum* by Mattocks (1). As a class, pyrrolizidine alkaloids have shown significant antitumor activity; however, most have associated toxic effects that would prevent clinical application. In preclinical tests, INDI has been active in various animal tumor systems with less toxicity than was found with other pyrrolizidine alkaloids.[4] The exact mechanism of action has not been determined for INDI; however, based on findings with other pyrrolizidine alkaloids, it may involve metabolic formation of chemically reactive intermediates that bind covalently with macromolecules (2). A phase I trial completed at this institution demonstrated hematologic dose-limiting toxicity and suggested increased myelosuppression in patients who had had prior exposure to a nitrosourea. The phase I data suggested a starting dose of 2250 mg/m²/day iv daily for 5 days every 5 weeks for patients with prior nitrosourea exposure and 3000 mg/m²/day given in a similar manner for patients with no prior exposure to chemotherapy or with exposure to chemotherapy other than a nitrosourea.[5] This report summarizes the therapeutic activity of INDI in advanced colorectal cancer using the intermittent 5-day schedule and describes the unpredictable toxicity encountered.

MATERIALS AND METHODS

Patients

Thirty patients with measurable, histologically proven, recurrent or metastatic colorectal adenocarcinoma participated in this study. All patients were acceptable candidates for cytotoxic chemotherapy, with a minimum wbc count of 4000/mm³ and a platelet count of 130,000/mm³, an Eastern Cooperative Oncology Group performance score of 0–3, and a satisfactory nutritional status. A serum bilirubin < 3.0 mg/dl and a serum creatinine < 2.0/100 ml were required prior to entry in the study. Fifteen patients had received prior chemotherapy with the following agents given alone or in combination: methyl-CCNU (seven patients); chlorozotocin (four); 5-FU (five); cyclophosphamide, doxorubicin, and cisplatin (two); and alanosine, DON, ICRF-159, triazinate, PALA, and maytansine (one each). A total of 11 patients had exposure to prior nitrosourea chemotherapy.

Drug Administration

Ampules (1-g) of lyophilized INDI were reconstituted with sterile water and were infused iv over 10 minutes daily for 5 days every 5 weeks. The first 11 patients entered in the study had had prior nitrosourea exposure and were treated at the dose level of 2250 mg/m²/day for 5 days. Eight of these 11 patients developed severe myelosuppression. After this initial experience, the study was closed to prior nitrosourea-treated patients; however, unpredictable myelosuppression in patients with either no prior chemotherapy or minimal prior treatment was also encountered at the 3000-mg/m²/day dose level. The remaining 11 patients with no prior chemotherapy were treated at a reduced dose of 2500 mg/m²/day for 5 days every 5 weeks.

Evaluation

Prior to initial treatment and each subsequent course, patients had a complete medical evaluation including the following laboratory studies: hemoglobin level; wbc and platelet counts; serum determinations of creatinine, bilirubin, alkaline phosphatase, SGOT, calcium, sodium, potassium, and uric acid; fasting plasma glucose; and a chest roentgenogram. After treatment, wbc and platelet counts were determined twice weekly. Patients who showed objective response or who remained stable were re-

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TABLE 1.—Hematologic toxicity following initial course of INDI

	No. of patients given—			
	Prior nitrosoureas (2250)*	Prior treatment, no nitrosoureas (3000)	No prior treatment (3000)	No prior treatment (2500)
Total	11	4	4	11
Wbc count nadir (cells/mm³)				
> 4000	3	1	—	3
4000–2500	3	1	3	5
2499–1000	2	—	—	2
< 1000	3	2	1	1
Platelet count nadir (cells/mm³)				
> 130,000	1	2	1	8
130,000–75,000	3	—	1	1
74,999–25,000	2	—	1	—
< 25,000	5	2	1	2
Hospitalized with drug-related complication	4	2	2	2

*Values in parentheses = INDI dose (mg/m²/day × 5).

treated at 5-week intervals until evidence of disease progression.

An objective response was defined as a ⩾ 50% decrease in the product of the longest perpendicular diameters of at least one clearly measurable indicator lesion. If hepatomegaly was the only measurable tumor indicator, there must have been a decrease of ⩾ 30% in the sums of measurements below the xiphoid process and each costal margin at the midclavicular lines. In addition, no new areas of malignant disease would have appeared. Progressive disease was defined as a > 25% increase in any indicator lesion. Objective stability was defined as the absence of either regression or progression.

RESULTS

Therapeutic

No patient satisfied the criteria for an objective response. Seventeen of the 30 patients (57%) had disease progression after only one cycle of treatment. Seven received two cycles, three received three cycles, one received four cycles, and only two presently remain stable after five and seven cycles of treatment. These two patients have shown some regression of pulmonary nodules, insufficient to meet our criteria for objective response.

Toxicity

Nonhematologic toxicity was mild. Of 60 evaluable courses of treatment, moderate nausea occurred in 12 (20%) with moderate vomiting acutely occurring in only six (10%). There was no evidence of alopecia or stomatitis, and all diarrhea, constipation, and anorexia seemed to be related to the patients' cancer.

Hematologic toxicity proved to be a major problem (table 1). Eight of the initial 11 patients with prior nitrosourea exposure developed significant neutropenia and/or thrombocytopenia. Four of the eight patients required hospitalization. One patient with a platelet count nadir of 20,000/mm³ experienced a cerebral vascular accident; one patient was hospitalized with a platelet count of 5000/mm³ and received periodic transfusions for 11 days until recovery. Two patients died; each had progressing tumor complicated by severe thrombocytopenia without evidence of significant bleeding.

Four of the eight patients treated at the 3000-mg/m²/day dose level developed thrombocytopenia requiring platelet transfusion; one patient also developed sepsis with a wbc count nadir of 300/mm³ requiring antibiotics. The other four patients treated at the 3000-mg/m²/day dose level experienced no significant myelosuppression. Only two of the last

11 patients treated at the 2500-mg/m²/day dose level who had had no prior chemotherapy developed severe myelosuppression. One patient required platelet transfusion while the other died from septic death with rapidly progressive tumor and severe marrow depression.

DISCUSSION

In this phase II study, patients with colorectal adenocarcinoma received INDI in a 5-day iv schedule intermittently at three different dose levels depending on their prior chemotherapy exposure. No objective responses occurred among 15 previously untreated and 15 previously treated patients. There was occasional mild gastrointestinal toxicity with no suggestion of hepatic toxicity. Even though patients treated with prior nitrosoureas were more sensitive to the myelosuppressive effects of INDI, the myelosuppression for all patients was unpredictable and often severe. Severe myelosuppression was not a function of age, performance status, amount of disease, or hepatic involvement.

The unpredictability and severity of the myelosuppression suggest to us the possibility of individual differences in activation or metabolism of the drug. From our experience, we conclude that INDI used in the intermittent 5-day schedule is inactive in advanced colorectal cancer, and we cannot recommend its use in this disease.

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Phase II Evaluation of AMSA in Adult Sarcomas[1,2]

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SUMMARY

AMSA, an acridine derivative, was administered to 35 adults with previously treated advanced sarcomas. Patients with adequate bone marrow reserve received 120–150 mg/m² of AMSA as a single dose repeated every 3 weeks. Patients with inadequate bone marrow reserve received 100–120 mg/m² of AMSA. Among 31 evaluable patients, there was one partial response that lasted 6 months in a patient with intra-abdominal malignant fibrous histiocytoma with liver metastases. Thirteen patients had stable disease with a median time to disease progression of 5 months (range, 2–13), while 17 patients demonstrated progressive disease with a median time to disease progression of 2 months (range, 1–3). The median survival time for the 31 evaluable patients in this study was 5 months. The toxic effects were mild and included myelosuppression, nausea and vomiting, fever of unknown origin, and fatigue. At the dose and schedule used in this study, AMSA does not appear to have any significant activity in advanced sarcomas of adults.

[Cancer Treat Rep 65:341–343, 1981]

AMSA is an acridine derivative that was synthesized in an effort to design drugs that would bind to DNA (1). It is active in a number of transplantable animal tumor systems and was selected for clinical trial because of its superior activity in the B16 melanoma system and its possible superior activity against liver metastases since it concentrates in bile (1). Phase I studies have indicated that the dose-limiting toxic effect of AMSA was myelosuppression (2,3). Antitumor activity was reported against adenocarcinoma of the lung and the ovary and against melanoma, lymphoma, and leukemia (2,3). In this paper, we report the results of a phase II evaluation of AMSA in adults with advanced sarcomas.

PATIENTS AND METHODS

Patients with biopsy-confirmed diagnoses of soft tissue or bony sarcoma, with measurable metastases, who failed regimens of higher priority were eligible for this study. All patients were required to have an absolute granulocyte count of $\geq 2000/\mu l$, a platelet count of $> 100,000/\mu l$, and normal renal and hepatic functions as measured by serum creatinine, BUN, SGOT, and bilirubin levels. These patients had received a median of two prior chemotherapeutic regimens, including a doxorubicin-DTIC regimen (range, one to eight), and the median duration of prior chemotherapy was 15 months (range, 2–52).

AMSA was supplied by the Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The starting dose for patients with adequate bone marrow reserve was 120 mg/m², in 250 ml of 5% dextrose in water over 1 hour. This dose was repeated every 3 weeks. For patients with inadequate bone marrow reserve, the starting dose was 100 mg/m² iv every 3 weeks. The starting doses were subsequently increased to 150 mg/m² for patients with adequate bone marrow reserve and to 120 mg/m² for those with inadequate bone marrow reserve. The dose in subsequent courses was modified by the degree of myelosuppression during the previous course. All patients were required to have weekly cbcs; liver and renal function tests and appropriate radiologic studies were repeated every 3–6 weeks. Tumor measurements were recorded every 3 weeks.

An adequate trial was defined as a minimum of

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TABLE 1.—Response by histologic type

Histologic type	No. of patients	Response		
		Partial	Stable disease	Progressive disease
Soft tissue sarcomas				
Leiomyosarcoma	7	0	3	4
Malignant fibrous histiocytoma	6	1	1	4
Unclassified	4	0	2	2
Liposarcoma	3	0	3	0
Synovial cell sarcoma	2	0	0	2
Breast sarcoma	2	0	0	2
Neurogenic sarcoma	1	0	1	0
Angiosarcoma	1	0	1	0
Bone sarcomas				
Osteogenic sarcoma	3	0	0	3
Chondrosarcoma	2	0	2	0
Totals	31	1	13	17

two courses of AMSA unless objective tumor progression occurred after one course. Response was assessed using standard criteria as previously described (4).

RESULTS

Of the 35 patients entered in this study, four were not evaluable for response (one patient died of advanced disease on Day 9 of the first course of AMSA, two were lost to followup after having received one dose of AMSA, and one refused further therapy after the first course of treatment). Of the 31 evaluable patients, there were 18 men and 13 women, with a median age of 45 years (range, 17–76).

The response of the various histologic types is shown in table 1. One patient achieved a partial response that lasted 6 months with marked subjective improvement. She had an intra-abdominal malignant fibrous histiocytoma with liver metastases. The median time to disease progression for the 13 patients with stable disease was 5 months (range, 2–13), and the median time to disease progression for the 17 patients with progressive disease was 2 months (range, 1–3). The median survival for the 31 evaluable patients in this study was 5 months (range, 1–22+).

The toxic effects of AMSA were predominantly hematologic and were dose-related (table 2). The starting dose for the first 19 patients was 120 mg/m^2 for those with adequate bone marrow reserve and 100 mg/m^2 for those with inadequate bone marrow reserve. Since no responses were observed at these dose levels, the next 12 patients received higher doses despite myelosuppressive toxicity. The single partial responder had received a starting dose of 150 mg/m^2. The blood cell counts of most patients returned to normal by Day 21, and there was no evidence of cumulative myelosuppression with repeated courses of treatment up to a maximum of 16 courses in one patient. Other side effects of AMSA were generally mild and consisted essentially of nausea and vomiting, which occurred in 21% of the courses. Three patients had fever of unknown origin associated with neutropenia, and two patients reported fatigue following treatment with the drug.

DISCUSSION

AMSA was selected for phase II clinical trial because of evidence of antitumor activity in a wide spectrum of tumors in phase I studies and also because its toxicity was generally mild, predictable, and reversible (2,3). In addition, a number of phase II studies have demonstrated that AMSA is active in acute leukemia, breast cancer, and melanoma (5,6). The current study was undertaken to determine whether AMSA had activity in the treatment of metastatic sarcomas.

The results of this trial in patients with sarcoma were disappointing because only one partial response was observed. Although all patients had received extensive prior chemotherapy, the performance status of most patients was good. Thus, it appears that AMSA, with the doses and schedule used in this study, has a very limited role in the

TABLE 2.—Hematologic toxicity

Dose (mg/m²)	No. of evaluable courses	Lowest counts* for — Wbcs Day	Wbcs Median	Wbcs Range	Polymorphonuclear neutrophils Day	Polymorphonuclear neutrophils Median	Polymorphonuclear neutrophils Range	Platelets Day	Platelets Median	Platelets Range
100	13	14	3.5	1.0–4.3	14	1.7	0.6– 30	14	170	44–236
120	24	14	2.4	1.2–5.1	14	1.0	0.1–3.5	12	140	57–412
150	26	14	1.5	0.4–3.6	14	0.7	0.2–1.8	14	119	28–300
170	3	12	1.0	0.1–1.1	12	0.6	0–0.6	11	128	7–224

*Median and range values = cells × $10^3/\mu l$.

treatment of patients with metastatic sarcomas, particularly of the soft tissues.

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Treatment of Advanced Colorectal Carcinoma With AMSA[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

AMSA in a dose of 120 mg/m² iv every 4 weeks was evaluated in 26 patients with advanced measurable colorectal cancer, 13 of whom had had no prior chemotherapy. The schedule was tolerated well except by those patients who had prior irradiation or chemotherapy, in whom a dose reduction to 90 mg/m² was required. No significant antitumor response was observed.

[Cancer Treat Rep 65:345–347, 1981]

This study was undertaken to evaluate the tolerance and effectiveness of AMSA in patients with advanced colorectal carcinoma since conventional chemotherapy is inadequate and the effects of AMSA were unknown.

AMSA is one of a series of acridine derivatives synthesized by Cain and Atwell (1) as potential antitumor agents. AMSA appears to act as an inhibitor of DNA synthesis and inhibits incorporation of labeled thymidine into the DNA of L1210 cells in vivo or in tissue culture. It is active in a variety of experimental tumor systems including L1210 and P388 leukemias, Lewis lung carcinoma, B16 melanoma, and both the mouse mammary and mouse colon tumors (2,3). Using a variety of schedules, antitumor activity in man has been reported in ovarian carcinoma, breast cancer, lymphoma, melanoma, adenocarcinoma of the lung, and acute myeloblastic leukemia (4,5).

Toxicology studies in beagle dogs and rhesus monkeys have demonstrated dose-related but reversible marrow hypoplasia, anorexia, vomiting, diarrhea, and weight loss. Atrophy and necrosis of lymph nodes and spleen were also noted. Toxic effects in man consist of leukopenia and, less commonly, thrombocytopenia, vomiting, and phlebitis (4,5).

MATERIALS AND METHODS

Patient Selection

Twenty-six patients with histologic evidence of metastatic colorectal carcinoma were studied. Thirteen patients had failed previous chemotherapy with 5-FU and/or nitrosoureas, and 13 had no previous chemotherapy. All patients had measurable disease and all had adequate bone marrow, renal, and hepatic functions. Sixty-one percent of patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (ambulatory), and 39% had a performance status of 2 or 3. None with a performance status of 4 (bedridden) were included.

Treatment Schedule and Study Parameters

AMSA was administered at a dose of 120 mg/m² in 500 ml of 5% dextrose in water and was given iv over 60 minutes. The dose was repeated every 4 weeks. Early experience during the trial led to reduction of the initial dose to 90 mg/m² in patients who had received prior cytotoxic or radiation therapy.

Pretreatment studies included history and physical examination, cbc with differential and platelet counts, SMA-12, serum electrolytes, BUN and creatinine, urinalysis, chest roentgenogram, and additional radiologic studies and radionuclide scanning as indicated. Cbc with differential and platelet counts, along with SMA-12, were obtained prior to each treatment and every 2 weeks thereafter.

Response criteria were as follows: *complete response*—the disappearance of all clinically detectable tumor; and *partial response*—at least a 50%

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TABLE 1.—Characteristics of treated patients

Characteristics*	Prior chemotherapy	No prior chemotherapy
No. of patients entered	13	13
Age in years		
Median	58	65
Range	44–77	52–80
ECOG performance status		
0/1	6	9
2/3	7	4
Predominant sites of metastasis		
Liver	5	3
Lung	5	7
Nodes and/or soft tissue	3	3
Average No. of treatments	2.2	2.6
Average dose in mg	205	204

*Unless otherwise indicated, values = No. of patients.

reduction of the product of the longest perpendicular diameters of the most clearly measurable lesions. If hepatomegaly was the primary indicator, there had to be at least a 30% reduction in the sum of the liver measurements below the costal margin at the midclavicular line and at the xiphoid process.

RESULTS

The characteristics of the 26 patients studied are summarized in table 1.

There were two early deaths from toxicity and one patient was lost to followup. Among the remaining 23 patients, there was one partial response. This occurred in an anterior abdominal wall mass in a patient previously treated with 5-FU and methyl-CCNU. The reduction in tumor size lasted 46 days and was not associated with clinical benefit since an enlarged liver did not shrink and performance status worsened during treatment.

Progression of disease has occurred in 21 patients, with a median time to progression of 63 days. Twenty-two of the 25 patients who had followup have died, with a median survival time for all patients of 6.3 months (range, 13–302+ days). Two patients died with clinically stable disease.

Toxicity

Table 2 shows the frequency and severity of side effects associated with AMSA. During the early stages of this trial, three patients who had been heavily pretreated with radiation and/or chemotherapy experienced life-threatening hematologic toxic effects and two died. Subsequent doses of AMSA in such patients were decreased to 90 mg/m² every 4 weeks. Using this dose modification where indicated, only one further episode of severe myelosuppression occurred in 47 courses of treatment. Eleven additional patients experienced mild-to-moderate leukopenia, but only five patients had platelet counts < 100,000/mm³, and only two had platelet counts < 50,000/mm³. Nadir values usually developed at 10–14 days after treatment and returned to normal by Day 28. Nausea and vomiting occurred in only two patients (8%). Two patients had low-grade fever during the 48 hours following drug administration. Ototoxicity and neurotoxicity were not observed. Phlebitis, a troublesome problem in earlier trials, was all but eliminated by diluting the drug in 500 ml of 5% dextrose in water and administering it over 60 minutes. No definite instances of liver toxicity were noted.

TABLE 2.—Summary of toxicity occurring in 26 patients

Toxic effect	No. of patients
Hematologic	
Leukopenia	
Wbc count < 1000/mm³*	4 (2 deaths)
Wbc count of 1500–3999/mm³†	11
Thrombocytopenia	
Platelet count < 100,000/mm³	5
Platelet count < 50,000/mm³	2
Mild nausea and vomiting	2
Fever	2

*Severe.
†Moderate.

DISCUSSION

This dose schedule of AMSA failed to demonstrate significant antitumor effects in patients with advanced colorectal cancer. Toxicity from a dose of 120 mg/m² every 4 weeks was acceptable, but we recommend that doses be reduced to 90 mg/m² in patients who have received radiotherapy (especially to the pelvis) or extensive chemotherapy.

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Combination of Vinblastine and Bleomycin in Metastatic Renal Cell Carcinoma

Renal cell carcinoma has been particularly resistant to chemotherapy. Clinical trials utilizing anthracyclines, antimetabolites, antimitotics, and alkylating agents have produced response rates varying from 0 to 20% (1–4). More recently, vinblastine, as a single agent, has been shown to give responses in the range of 13%–30% (1–3). Bleomycin is another agent that has shown mixed results in phase II studies; as a single agent, it has given partial response rates of 0–35% (2,3).

In vitro data on these two agents have demonstrated that vinblastine is a potent spindle cell poison arresting cells in mitosis, and bleomycin causes maximum tumor-kill in the late G_2 and mitosis phase. With such theoretic considerations in mind, several investigators have sequenced vinblastine and bleomycin in an attempt to take advantage of these mechanisms (2). This study was designed to look at the combined administration of vinblastine and bleomycin, and to take advantage of any additive or synergistic effect in renal cell carcinoma.

MATERIALS AND METHODS

Fifteen patients (two females and 13 males) with biopsy-proven metastatic renal cell carcinoma were studied. They ranged in age from 43 to 73 years. The predominant sites of disease were lung and bone; other organs involved were liver, skin, and brain. Performance status varied from 50% to 100%. Three patients had received prior chemotherapy with methotrexate, CCNU, or Baker's antifol. Only one patient had received prior endocrine therapy with medroxyprogesterone acetate.

Our regimen consisted of weekly vinblastine (0.2 mg/kg iv) followed 2 hours later by bleomycin (15 units/m^2 iv). Bleomycin was given to a total dose of 400 units as long as the patient was stable or responding and there was no clinical evidence of pulmonary toxicity.

Objective tumor response was determined by serial measurements. The grading of response was as follows: *complete response*—the total disappearance of all measurable disease; and *partial response*—a $\geq$ 50% decrease in the products of the maximum perpendicular diameters.

RESULTS

Of the 15 patients, two achieved a partial response. The partial responses were of short duration, lasting 4 and 5 months. Since the conclusion of this study, all patients have died of their disease.

The primary toxic effect of this regimen was leukopenia. Of 185 weekly treatments with vinblastine, the dose was maintained 30 times. The lowest wbc count recorded was 1100/mm^3. There were no infections directly related to the leukopenia. No dose adjustments were required because of thrombocytopenia. No pulmonary toxicity was noted in any of the patients, and three of 15 received 400 units of bleomycin.

DISCUSSION

Approximately 12,000 individuals will develop renal cell carcinoma each year in the US, and one third of these will present initially with metastatic disease. The natural history of untreated disease is dismal, with 4.4% of the patients surviving 3 years and 1.7% alive at 5 years (5). Hrushesky and Murphy (2) analyzed the clinical trials with vinblastine used alone and in combination. As a single agent, a total of 135 patients were treated with doses of 0.1–0.3 mg/kg. Of this group, three patients achieved complete responses, 23 achieved partial responses, and seven had stabilization of disease; the overall response rate was 25%. Our results showed two of 15 patients responding (a 13% response rate). This combination does not appear to show any advantage over single-agent therapy.

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Cerebrospinal Fluid Levels of Hexamethylmelamine and N-Demethylated Metabolites[1]

Only a limited number of anticancer agents cross the blood-brain barrier and can be used for the treatment of CNS primary tumors or metastases. The anticancer agent hexamethylmelamine (HMM) has shown activity in some human malignancies (1), particularly ovarian cancer and small cell lung carcinoma. Since the latter often metastasizes to the CNS, it would be interesting to know whether HMM and its active metabolites are present in cerebrospinal fluid (CSF) at effective concentrations.

Knowledge of CSF levels might also offer some explanation of the CNS toxicity which can occur after HMM treatment (2), and appears to be a use-limiting drawback of pentamethylmelamine (PMM) which is under investigation in phase I clinical trials.[2]

In a 51-year-old patient with gestational choriocarcinoma (with intestinal and lung metastases) who underwent lumbar puncture for diagnostic (measurement of CSF β-human chorionic gonadotropin levels) and therapeutic (administration of methotrexate intrathecally) purposes, CSF and plasma concentrations of HMM and some metabolites were determined after a single oral dose of 130 mg/m².

The patient had no apparent CNS metastases at the time of the study and did not develop them subsequently.

The patient gave her informed consent for this study. At 230 mins after HMM administration 5 ml of CSF was taken and was replaced immediately with 5 ml of sterile water containing 10 mg of MTX. Blood samples were taken before HMM administration and at intervals up to 24 hours including the time at which CSF was taken. CSF or plasma (0.5 ml) was extracted at pH 11.5, once with *n*-hexane and once with ethylacetate, and a previously described (4) gas-liquid chromatography method with nitrogen detector was applied.

Figure 1 shows the plasma level time course of HMM, PMM, $N_2N_2N_4N_6$-tetramethylmelamine

[1]Supported by CNR contract 78.02849.96.

[2]Annual report: pentamethylmelamine (NSC-118742, IND 14585). Prepared by the National Cancer Institute, Bethesda, MD, Sept 1979.

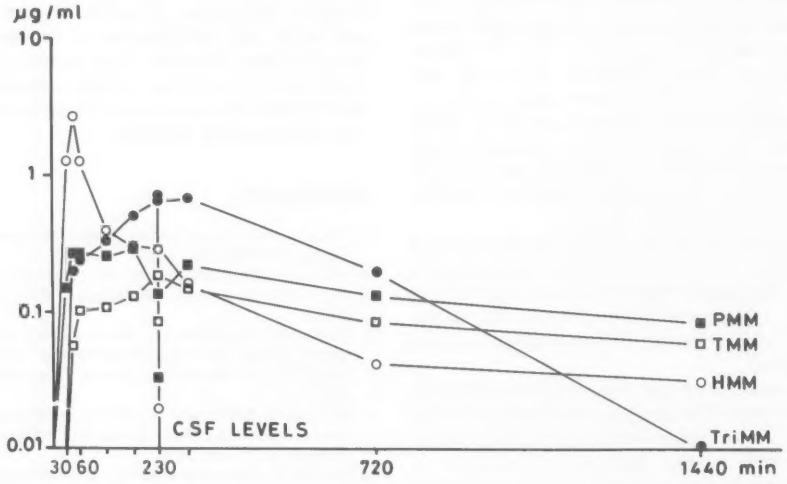

FIGURE 1.—Plasma levels of HMM, PMM, TMM, and TriMM at intervals after HMM treatment. CSF levels are indicated along the vertical line at 230 mins.

(TMM), $N_2N_4N_6$-trimethylmelamine (TriMM), and the concentrations in CSF 230 mins after HMM administration. CSF concentrations of HMM, PMM, TMM, and TriMM amounted to 6%, 24%, 47%, and 100%, respectively, of those in plasma. These differences are probably due to different plasma protein binding of these compounds. The free plasma protein fractions of HMM, PMM, and TMM, determined by equilibrium dialysis at 37°C, were, in fact, 6%, 25%, and 50%, respectively. Protein binding of TriMM has not yet been studied.

On the basis of these data it is reasonable to hypothesize that the metabolites of HMM could play a role in the neurotoxicity observed after HMM treatment. This is consistent with the clinical observation that PMM causes much more severe CNS side effects than the parent compound. It is also likely that the levels of HMM or its metabolites in CSF are low compared to those necessary to induce a cytotoxic effect in vitro (4), unless there is some summation of activity.

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Avoidance of Renal Toxicity by 24-Hour Infusion of Cisplatin

Cisplatin is usually administered as a single iv dose of 50–75 mg/m² or as five daily iv doses of 15–20 mg/m². Hydration with or without mannitol-induced diuresis is recommended to minimize nephrotoxicity (1). Recently, Jacobs et al (2) reported on the use of 24-hour infusion of cisplatin in the treatment of advanced head and neck cancer. The renal toxicity of this method was minimal. This may have been due to low plasma platinum levels resulting from a prolonged infusion rather than a bolus method.

Table 1.—Creatinine clearance and plasma creatinine levels

Cycle No.	Mean creatinine clearance ± SE (ml/min)	Mean plasma creatinine ± SE (mg/100 ml)
0	83.4 ± 5.4	0.81 ± 0.04
1	77.4 ± 6.5	0.79 ± 0.04
2	82.6 ± 5.6	0.79 ± 0.04
3	83.3 ± 6.8	0.76 ± 0.04
4	77.6 ± 11.2	0.94 ± 0.13

We have now treated 22 patients with stage III and IV ovarian carcinoma using 60 mg/m² of cisplatin in a 24-hour continuous iv infusion. The minimum interval between infusions was 3 weeks.

There is controversy on the stability of cisplatin in low chloride concentration and on exposure to light (3,4). We thus chose 5 liters of normal saline as the total volume to be infused over 24 hours and then divided the dose of cisplatin (usually 100 mg) equally so that about 10 mg in 500 ml of normal saline was infused in 2.4 hours. Also, the infusion bag and administration set were covered to prevent exposure to light, but the drip chamber was left accessible to inspection by the nursing staff.

A total of 82 infusions have been given. Most patients have had four courses, with a range from one to seven. Renal function was monitored by serial serum creatinine levels and creatinine clearances. The results are shown in table 1.

Response was assessed by clinical examination and by serial ultrasonic scans where the pretreatment scan had shown an abnormality.

Of the 22 patients treated, 14 responded. Ten of these patients have subsequently relapsed. Eight patients failed to show any response. Of these eight patients, seven had received previous treatment with alkylating agents, whereas none of the four patients still in remission had had previous therapy.

We conclude that this method of administering cisplatin is safe at the dose level used. Although the diuresis induced by 5 liters of fluid in 24 hours could by itself be responsible for the lack of toxicity, there is a theoretic basis for supporting that cisplatin could be less toxic and more effective when administered in a prolonged infusion (5). We are at present treating patients with an increased dose of 80 mg/m² and have reduced the total infusion volume to 3 liters of normal saline.

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[1]We thank the National Cancer Institute, Bethesda, MD, and Mead Johnson Limited, for initial supplies of cisplatin. We are also grateful to the consultant gynecologists who referred these patients and to the nursing staff for their cooperation in treating them.

Cutaneous Necrosis From Chemotherapy in a Patient With an Arteriovenous Fistula

Arteriovenous anastomoses facilitate long-term iv therapy for a variety of hematologic and neoplastic conditions. Complications of vascular access devices include infection, thrombosis, "steal" syndromes, bleeding, and congestive heart failure (1–4). We have recently seen an unusual complication of chemotherapy in a patient with an arteriovenous fistula.

CASE REPORT

A 43-year-old man with a history of parenteral drug abuse developed warm IgM autoimmune hemolytic anemia in 1978. Treatment with prednisone and splenectomy and multiple-drug therapy with cyclophosphamide, vincristine, and prednisone were only transiently effective. In September 1979, he was severely symptomatic, with a hemoglobin of 6.3 g/100 ml and a reticulocyte count of 12%. Vinblastine was begun as a weekly 8-hour infusion (5), with excellent response.

A primary arteriovenous fistula of the left brachial artery and the left basilic vein, created for

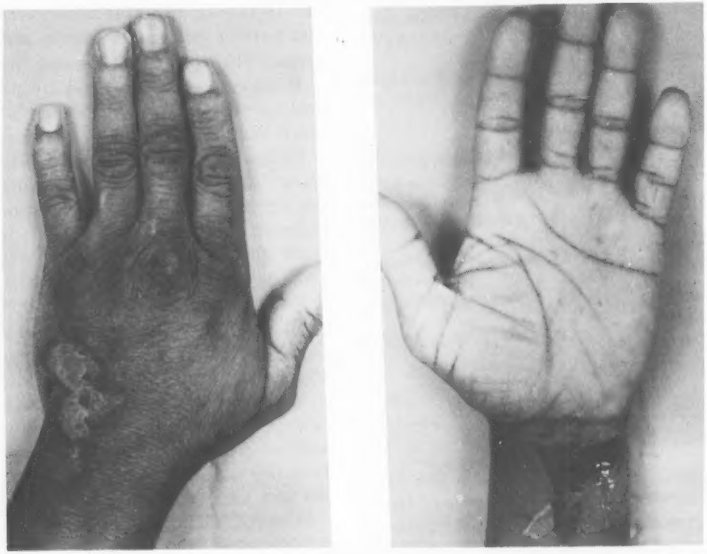

FIGURE 1.—Skin necrosis which developed following vinblastine infusion into a superficial vein. Dorsal and ventral views were taken several wks after injection.

long-term vascular access in 1978, remained unused because superficial veins became more prominent following surgery. In October 1979, vinblastine was infused through a superficial vein distal to the shunt. There were no signs of extravasation; however, cutaneous necrosis developed (fig 1). An angiographic study was performed, and partial obstruction of the fistula was observed. Contrast material injected into the venous portion of the fistula flowed retrograde into the superficial veins of the forearm and then to the deep venous system. The necrotic areas resolved over 6 months and all further chemotherapy has been delivered proximal to the fistula.

DISCUSSION

In the present case, incomplete venous obstruction of an arteriovenous fistula led to retrograde flow in superficial arm veins. The resulting perfusion of small cutaneous vessels with vinblastine resulted in tissue necrosis. Guba et al (3) noted that a major reason for late malfunction of arteriovenous fistulas is pseudointimal proliferation of the venous side of the structure. Such proliferation, which results in decreased blood flow and promotes thrombosis, may have occurred in our patient.

This case suggests that incomplete obstruction of arteriovenous connections may not be readily apparent and may cause serious tissue loss when sclerosing chemotherapeutic agents are administered. Injection into superficial veins distal to malfunctioning shunts may be particularly prone to complication, and caution is required even when long-standing, well-functioning shunts are used.

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Chronic Urticarial Reactions Caused by Doxorubicin-Containing Regimens

Doxorubicin has come into wide use as a cancer chemotherapy agent since its clinical introduction in 1969. Urticarial reactions to this drug have been reported as rare occurrences (1–3). Most commonly reported are erythematous and pruritic urticarial reactions appearing along the course of the vein for several centimeters proximal to the venipuncture site during drug infusion (1–3). These reactions are usually transient, do not necessarily appear with every treatment, and improve either spontaneously or with antihistamines. Generalized urticarial reactions without anaphylaxis have also been noted by authors on the day of treatment (1,3). However, anaphylaxis has also been reported (1). This report describes two patients who developed more chronic urticaria than previously reported while receiving doxorubicin-containing chemotherapy regimens for metastatic breast carcinoma.

CASE REPORTS

Patient 1

In April 1977, a 47-year-old white female was found to have widespread breast cancer with metastases to skin, bone, bone marrow, liver, and lungs. She took no medications regularly. Medical history revealed an allergy to sulfonamides which had caused a rash. The patient began to receive reduced doses of chemotherapy consisting of 40 mg of doxorubicin given iv on Day 1, followed by 200 mg of cyclophosphamide given orally daily for 4 days beginning on Day 3, as reported by Jones et al (4). Doses were escalated to the tolerance of her marrow involvement and she received two cycles without incident. However, at the beginning of each of the third and fourth cycles, she experienced generalized, transient urticaria which spontaneously subsided on the day of treatment. At the fifth cycle of treatment, the patient received 55 mg of doxorubicin, bringing the cumulative dose to 245 mg. Throughout the following month the patient experienced intermittent attacks of urticaria which responded poorly to diphenhydramine hydrochloride (Benadryl). After receiving doxorubicin on Day 1 of the seventh course of treatment, an urticarial

reaction was sufficiently severe to cause the patient to seek help from her local hospital emergency room. She was given ACTH gel and epinephrine and achieved partial relief. She was admitted to our hospital the following day free of skin lesions. That night, showers of urticaria were observed and required sc epinephrine for control. Control of her urticarial attacks was finally achieved with dexchlorpheniramine maleate (Polaramine Repetabs). The patient was readmitted for the subsequent treatment with doxorubicin and again experienced urticaria within 24 hours of administration and for several weeks thereafter. The urticarial attacks subsided spontaneously just prior to the next chemotherapy cycle, when they would return with treatment. Later cycles were given on an outpatient basis, with the patient controlling her urticarial episodes with antihistamines. The patient ultimately completed 15 cycles of therapy, receiving 760 mg of doxorubicin (540 mg/m^2), and her chemotherapy program was switched to a combination of cyclophosphamide, methotrexate, and 5-FU (4). She had no further episodes of urticaria after the doxorubicin was discontinued.

Patient 2

In February 1977, a 64-year-old white female with breast carcinoma metastatic to bone and soft tissue was examined. Hormonal therapies had been used initially. Regular medications included Dyazide for hypertension and acetominophen. Previous allergic history revealed an intolerance to Darvon Compound and an allergy to betalin complex evidenced by a rash. Secondary to a worsening bone scan and chest roentgenogram, in May 1979, the patient began to receive a chemotherapy regimen including doxorubicin (25 mg iv) on Days 1 and 8, 5-FU (500 mg iv) on Days 1 and 8, and cyclophosphamide (75 mg orally) on Days 1–14, on a 28-day cycle (5). Initial doses were reduced secondary to leukopenia and recent radiation therapy. The patient received two complete cycles without incident. No dose escalation was possible because of bone marrow intolerance. At the beginning of the third cycle, she experienced generalized urticaria 4 hours after the treatment. Complete control was established within 24 hours with diphenhydramine hydrochloride (Benadryl). The cumulative dose of doxorubicin was 125 mg at this point. The following week generalized urticaria again appeared spontaneously at the time of the doxorubicin infusion. Control was achieved within 24 hours with Benadryl.

On Day 3 of the fourth cycle the patient had another urticarial episode requiring both Benadryl and dexachlorpheniramine maleate (Polaramine Repetabs). This resulted in gradual improvement but required 10 days for complete resolution. On Day 17 of the cycle, urticaria again appeared, subsiding slowly with Benadryl and Polaramine. Cycle 5 found the patient taking Benadryl as needed for an occasional isolated hive. The patient has now received nine cycles of treatment and remains in a partial remission. There has been no urticaria recurrence for the last four cycles, and the patient has not required further Benadryl administration.

DISCUSSION

The observation of urticaria appearing periodically during therapy with doxorubicin-containing regimens in these two patients has several interesting aspects. First, although bothersome, neither patient developed more serious allergic reactions and both continued to receive therapy. Second, urticarial reactions developed only after two cycles of therapy and were, at first, immediate and transient in nature on the day of doxorubicin therapy. These transient reactions heralded the onset of generalized urticaria in later cycles. Third, both patients experienced improvement with antihistamine administration. We believe that doxorubicin is clearly the offending agent in patient 1; however, the urticarial reaction stopped spontaneously in patient 2 despite continuation of the doxorubicin-containing regimen. It is possible that another drug(s) might be implicated in patient 2. Medical personnel treating patients with doxorubicin-containing regimens should be aware of this potential drug-related complication.

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Phase II Study of Vindesine in Metastatic Malignant Melanoma[1]

The Eastern Cooperative Oncology Group (ECOG) has conducted a phase II trial of vindesine (1,2)[2] in 26 patients with metastatic malignant melanoma. Thirteen male and 13 female patients were entered in this study. The median age was 55 years (range, 31–77) and the median ECOG performance status was 1 (ambulatory with mild symptoms). Thirteen patients had received no prior chemotherapy or radiotherapy and three had received only single-drug prior treatment. In 21 patients evaluable for response, measurable disease was distributed as follows: skin and/or lymph nodes (ten patients); pulmonary metastases (six); skin and pulmonary metastases (three); liver metastases (one); skin, lymph nodes, lung, and liver metastases (one).

Vindesine was administered at a dose of 4 mg/m^2 as a weekly iv injection. Weekly doses were modified or withheld on the basis of leukopenia, thrombocytopenia, or hepatic or neurotoxic effects. Treatment was carried out for a minimum of six doses unless unacceptable toxicity or disease progression occurred.

All 26 patients were evaluable for toxicity; hematologic toxicity was dose-limiting and neurologic toxicity was also prominent. A summary of toxicity encountered appears in table 1.

Two partial responses were observed in 21 patients evaluable for response, for a response rate of 9.5%. One previously untreated (performance status 0: asymptomatic) patient had shrinkage of metastatic skin nodules over a 2-month period. A second patient previously treated with methyl-CCNU (performance status 2: bedridden < 50% of time) had disappearance of an abdominal mass, shrinkage of an enlarged liver, and disappearance of bone pain lasting 3 months.

This study does not confirm higher response rates of melanoma to vindesine reported by British studies (3,4). The use of vindesine on a weekly schedule is seriously limited by myelosuppression and progressive neurologic toxicity. As a single agent, vindesine has little prospect of improving the therapy for metastatic malignant melanoma.

[1]This study was conducted by the Eastern Cooperative Oncology Group (ECOG) (*Paul P. Carbone, Chairman,* CA-21115) and supported by Public Health Service grants CA-11083 (*J. C. Arseneau,* University of Rochester Cancer Center, NY), CA-10572 (*S. J. Mellette* and *M. Kuperminc,* Medical College of Virginia, Richmond), and CA-10948 (*J. Wolter,* Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[2]Vindesine: Compound 99094: desacetyl vinblastine amide sulphate. An Investigational New Drug, 1975. Supplied by Eli Lilly and Co, Indianapolis, IN.

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Table 1.—Toxicity of vindesine in 26 patients with malignant melanoma

	No. of patients				
	Degree of toxicity				
Toxic effect	0: none	1: mild	2: moderate	3: severe	4: life-threatening
Hematologic	7	4	9	6	0
Neurologic	13	4	9	0	0
Alopecia	16	0	7	3	0
Mucositis	21	1	2	2	0
Gastrointestinal	13	7	5	1	0
Pulmonary	26	0	0	0	0
Cardiovascular	26	0	0	0	0
Renal	26	0	0	0	0
Miscellaneous	23	2*	1†	0	0

*1 transient elevation of BUN; 1 transient elevation of SGOT.
†1 transient episode of chills and flushing following iv drug administration.

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Phase II Evaluation of PALA in Patients With Metastatic Lung Cancer[1]

PALA, an inhibitor of aspartate transcarbamylase, was selected for clinical trials because of activity against experimental tumor models (1,2). From phase I studies, dose-limiting toxic effects are primarily dermatologic and gastrointestinal.[2] Since a dose of 5 g/m²/2 weeks produced toxicity in approximately one third of patients, we elected to use a comparable dose at 3–4-week intervals in patients with squamous cell (SQC), large cell (LGC), adenocarcinoma (ACA), and small cell (SMC) carcinoma of the lung.

MATERIALS AND METHODS

Fifty-eight patients with measurable or evaluable biopsy-proven non-SMC lung cancer received PALA at a dose of 5–6 g/m²/2 hours repeated every 3–4 weeks (table 1). Patients with SMC lung cancer were also entered in the study if clinical or laboratory parameters prevented the use of known effective regimens. Six patients received the lower dose because of age > 77 years or performance score of 3 or prior chemotherapy.

RESULTS

Therapeutic.—Among 19 patients without prior chemotherapy, we observed no objective regressions. Among 38 patients with prior chemotherapy, we observed two regressions (5%) for 7 weeks and 20 weeks, respectively (3). Thirty-nine patients pro-

[1]Supported in part by contract N01-CM-57044 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[2]Minutes of the Phase I Working Group Meeting. Cancer Therapy Evaluation Program, National Cancer Institute, Oct 1978, Bethesda, MD.

TABLE 1.—Pretreatment characteristics of 57 patients treated with PALA

	No. of patients						
	No prior chemotherapy			Prior chemotherapy*			
	ACA-LGC	SQC	SMC	ACA-LGC	SQC	SMC	Total
Total	11	7	1	20	16	2	57
Measurable	3	1	0	10	6	1	21
Evaluable	8	6	1	10	10	1	36
Sex							
Male	6	7	1	13	13	2	42
Female	5	0	0	7	3	0	15
Age (yrs)							
40–49	0	1	0	1	2	0	4
50–59	1	0	0	7	4	1	13
60–69	5	2	1	9	5	1	23
70–79	5	3	0	3	5	0	16
80	0	1	0	0	0	0	1
Performance score†							
0–1	8	3	0	16	10	2	39
2–3	3	4	1	4	6	0	18
Regressions	0	0	0	1	1	0	2

*Doxorubicin, cisplatin-based polychemotherapy regimens.
†Performance score: 0 (fully active) to 4 (totally disabled).

gressed at the time of reassessment. Seventeen patients did not return because of apparent tumor-related deterioration. One patient was taken off study due to toxicity.

Toxicity.—Mild nausea, vomiting, and diarrhea affected 25% of patients. Approximately one third remarked about a transient, blotchy, erythematous, and pruritic rash primarily on the neck and upper trunk. Two patients developed severe polyarthropathy, especially of the hands, wrists, and knees within 48 hours of receiving PALA. Symptoms lasted approximately 7 days and were controlled with indomethacin.

DISCUSSION

The lack of apparent efficacy of PALA with our regimen is consistent with another study of patients with non-SMC lung cancer. Weekly doses of 3.75–4.5 g/m^2 resulted in no major objective regressions among 22 patients (4). From the schedule used in our study, we can reject at the $\alpha = 0.05$ level of significance the hypothesis that the 4-month objective tumor regression rate is at least 15% among comparable patients with advanced non-SMC lung cancer who have received no prior chemotherapy. Additional studies are necessary to clarify the role of PALA in patients with SMC lung cancer.

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Ifosfamide Treatment of Pancreatic Cancer

Chemotherapy for pancreatic carcinoma has been disappointing (1). We recently treated ten patients

TABLE 1.—Treatment results

Patient No.	Age/sex	Disease extent at surgery	No. of courses	Response*	Duration of response (mos)
1	56/M	Advanced, peritoneal nodules	12	PR	18
2	25/M	Epigastric mass, supraclavicular nodes	3	PR	8
3	52/M	Epigastric mass, jaundice	2	NR	0
4	48/M	Epigastric mass, peritoneal nodules	10	PR	12
5	52/F	Peritoneal nodules	4	PR	6
6	54/F	Abdominal mass, peritoneal nodules	3	NR	0
7	63/M	Abdominal mass, peritoneal nodules	3	PR	4
8	58/M	Abdominal mass, peritoneal nodules, jaundice	2	NR	0
9	50/M	Abdominal mass, peritoneal nodules, jaundice	5	PR	7
10	54/M	Abdominal mass, peritoneal nodules	2	NR	0

*PR = partial response; NR = no response.

with advanced, inoperable adenocarcinoma of the pancreas at the National Cancer Institute, Cairo, Egypt. All patients were explored surgically, where extent of disease was verified and diagnosis was established by biopsy. Ifosfamide was then given in 5-day courses in a daily dose of 2 g/m² administered as a 2-hour infusion. Vigorous oral and iv hydration were employed. Courses were repeated every 2 weeks. The results are shown in table 1. Response was detected clinically by measurements of palpable nodules and liver size and by estimates of abdominal mass size.

Six patients exhibited criteria for a partial response (reduction of tumor by > 50% of the product of two tumor diameters). Responses lasted up to 18+ months. All patients complained of dysuria, and hemorrhagic cystitis was noted in eight patients. Treatment was also accompanied by mild leukopenia, nausea, and vomiting in all patients.

The manufacturer (Asta-Werke, Germany) reports two complete and eight partial responses in 13 patients with pancreatic carcinoma treated with ifosfamide (2). We conclude from this small series that ifosfamide is active in the treatment of pancreatic carcinoma and deserves further trial.

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Phase II Evaluation of Cycloleucine in the Treatment of Patients With Disseminated Sarcomas[1]

While chemotherapy for disseminated sarcoma has improved since the introduction of doxorubicin, once clinical progression is manifested, there is a notable absence of worthwhile second-line chemotherapy (1). Previous trials in > 200 patients treated with cycloleucine have shown varying activity compared to doxorubicin (2,3).[2]

We developed a new treatment schedule for cycloleucine to incorporate the pharmacokinetic parameters that were developed (4). Fourteen consecutive patients with disseminated sarcomas that had previously been treated with doxorubicin-containing regimens received 40–52 g/m² of cycloleucine at 3-week intervals. The patient population consisted of seven men and seven women with a median age of 39 years. The median performance status was 67. Six patients had leiomyosarcoma, five had soft tissue sarcomas, and three had bony sarcomas. In 12 patients metastases were to visceral sites while two had recurrent regional masses. No patient experienced clinical benefit. Indeed, the median time to progression of this group was 5 weeks. Toxicity was of three types: central nervous system, myelosuppression, and gastrointestinal. Six patients had reversible neurologic toxic effects which included some or all of the following: light headedness, dizziness, lethargy, confusion, ataxia, and coma. Myelosuppression occurred in three patients with greater effect noted on the platelet count. Six patients had mild to moderate nausea and vomiting. We conclude that cycloleucine has insufficient activity in sarcomas to warrant further investigation.

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Therapy for Advanced Colorectal Cancer With a Combination of 5-FU and Anguidine: A Southwest Oncology Group Study[1]

Anguidine (diacetoxyscirpenol) was found to be active against mouse colon adenocarcinoma No. 38, and to potentiate the activity of 5-FU in this tumor when the two were used in simultaneous combination (1).

5-FU and anguidine were administered in a pilot study to 30 patients without prior chemotherapy who had advanced, measurable colorectal carcinoma. Patients were divided into good- and poor-risk groups based on age, prior therapy, and liver functions (2). Good-risk patients received a bolus of 350 mg/m² of 5-FU followed by 3.0 mg/m² of anguidine given by 4-hour infusion. Both drugs were given daily × 5, with courses repeated every 28 days. Poor-risk patients had the dose of 5-FU reduced to 300 mg/m² and the anguidine to 2.5 mg/m². Median age of all patients was 61 years, and 20 of 30 patients had liver metastasis.

Two of 26 (7.7%) evaluable patients achieved a partial response (95% confidence limits, 1.5–27), and five of 26 (19.3%) had stabilization (95% confidence limits, 5.5–40). The liver was the site of response in both patients. No poor-risk patient responded.

Median survival was 14+ months for partial responders, 8+ months for those with stable disease, and 6 months for those with increasing disease. Median survival of patients with increasing disease was 2 months if no further chemotherapy was given and 9 months if other combinations were given. All patients were evaluable for toxicity (table 1).

The results of this study were similar to those from M. D. Anderson Hospital and Tumor Institute with respect to response rates and toxic effects (3). Dose levels were similar in both studies.

Based on the results of these studies, it appears that the combination of 5-FU plus anguidine offers no advantage over 5-FU alone and may be more toxic.

Table 1.—Toxic effects in 30 patients treated with 5-FU and anguidine

Toxic effect	No. of patients
Leukopenia (cells/mm³) Median: 2400 Range: 600*–3800	19
Thrombocytopenia (cells × 10^3/mm³) Median: 83 Range: 25–99	14
Nausea and vomiting	11
CNS (hallucinations)	1
Pulmonary fibrosis	1*

*Fatal cases.

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Nominations for Bristol-Myers Chemotherapy Awards

The Scientific Committee of the International Society for Chemotherapy is requesting nominations for Bristol-Myers Chemotherapy Awards to be given for the first time during the Twelfth International Congress of Chemotherapy, July 14–17, 1981. Two awards will be given, amounting to $2000 each, plus travel. One award will honor outstanding contributions to the chemotherapeutic control of infectious disease; the other will be given for chemotherapeutic control of neoplastic disease. Nominations should cite the contributions of the nominee, and a curriculum vitae and bibliography should be enclosed. *Send nominations to*: Thomas C. Hall, MD, Scientific Committee, Cancer Center of Hawaii, 1236 Lauhala St, Honolulu, HI 96813.

Roswell Park Memorial Institute Continuing Education Programs

Buffalo, New York, September–December, 1981

Roswell Park Memorial Institute is conducting monthly continuing education programs in oncology for community practitioners to disseminate information on newest methods in the diagnosis and treatment of cancer. Each monthly seminar is organized by a different department of the Institute covering a different clinical specialty. These seminars qualify for Category I credit toward the AMA Physician's Recognition Award, prescribed credit by the American Academy of Family Physicians, and Category 2-D credit by the American Osteopathic Association. There is a $25.00 registration fee for one-day seminars and a variable fee for longer programs. The seminars are co-sponsored by the Cancer Control Program of the Institute and the American Cancer Society, New York State Division and Erie County Unit.

The seminars are scheduled as follows: Membrane Receptors, *September 17, 1981* (chairperson: Dr. Daryl Doyle); Nutrition and Cancer, *October 1–2, 1981* (chairperson: Dr. Fred Rosen); Controversies in the Management of Thyroid Cancer, *November 5, 1981* (chairperson: Dr. John Lore); Human Interferon, *December 10, 1981* (chairperson: Dr. Julius Horoszewicz).

Inquiries should be sent to: Gayle Bersani, RN, Cancer Control Coordinator, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263 (telephone: 716–845-4406).

Oral Complications of Cancer Chemotherapy

Baltimore, Maryland, September 21–22, 1981

A continuing education course entitled Oral Complications of Cancer Chemotherapy will be held September 21–22, 1981, in Baltimore, Maryland. The course is sponsored by the Sidney Farber Cancer Institute, Brigham and Women's Hospital, the Baltimore Cancer Research Program, and the Baltimore College of Dental Surgery. *For additional information contact*: Program of Continuing Education, University of Maryland School of Medicine, 10 S Pine St, Baltimore, MD 21201 (telephone 301–528-3956).

Third Breast Cancer Working Conference

Amsterdam, The Netherlands, April 27–29, 1983

The Third Breast Cancer Working Conference of the Breast Cancer Group, European Organization for Research on Treatment of Cancer, will be held April 27–29, 1983, at the RAI Congress Centre, Amsterdam. *For further information, contact*: Progam Committee Office, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, The Netherlands.

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